COMPARATIVE STUDIES OF THE REPRODUCTIVE STRATEGIES OF NEW ZEALAND GRAPSID CRABS (BRACHYURA: GRAPSIDAE) AND THE EFFECTS OF PARASITES ON THEIR REPRODUCTIVE SUCCESS

by

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Abstract

Comparative studies of the reproductive strategies of New Zealand grapsid crabs (Brachyura: Grapsidae) and the effects of parasites on their reproductive success

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The reproductive strategies of four intertidal grapsid crabs, *Hemigrapsus sexdentatus*, *H. crenulatus*, *Cyclograpsus lavauxi*, and *Helice crassa*, were studied in the field and laboratory, with emphasis on mating behaviour, duration of female receptivity, and sperm competition. Mating occurred in all species during the intermoult on the days prior to oviposition, when the gonopore opercula of females became temporarily mobile. Female *Helice crassa* mated up to three weeks after oviposition, but in all other species mating typically ceased at egg-laying. Male *Hemigrapsus* spp. used a female-centered competition strategy in which they searched for and defended receptive females until they laid eggs. In contrast, male *C. lavauxi* searched for and intercepted receptive females only for the duration of copulation and then pursued other receptive females (a mating system termed encounter rate competition with pure search and interception). Male *Helice crassa* searched for receptive females in their immediate neighbourhood and mated with them briefly on the substrate or in the burrow after which the female left (a mating system termed encounter rate competition with neighbourhoods of dominance). The mating season was short and highly synchronous for *Hemigrapsus sexdentatus* and *Cyclograpsus lavauxi* and asynchronous for *Hemigrapsus crenulatus* and *Helice crassa*. In the laboratory, the mean duration of receptivity for females housed with three males varied between 4.1 and 12.4 days, and the copulation frequency of females varied before oviposition between 2.1 and 24.3 times (mean) depending on the species. Female *Hemigrapsus* spp. isolated from males stayed receptive significantly longer than females held continuously with males. This suggests that females are able to control the duration of their receptivity, and therefore the time available for mating, according to the absence or presence of males. The operational sex ratio (OSR) had no effect on the duration of female receptivity, but female *Hemigrapsus crenulatus* mated more often when several males were competing for access. Therefore, male-male competition increased the number of matings per female and hence sperm competition within the female spermathecae. Larger males mated significantly more often than smaller males in all species. However, male size did not affect ejaculate size, meaning that small and large males transferred similar-sized ejaculates, e.g., in *Hemigrapsus* spp. Males of the two *Hemigrapsus* species followed a different strategy of sperm allocation. Male *H. crenulatus*,

which are typically confronted with a high mating frequency of the female and a long, asynchronous mating season, distributed similar-sized ejaculates, irrespective of female size. By contrast, male *H. sexdentatus*, which experience a comparatively lower risk of sperm competition during a short, synchronised mating season, invested larger ejaculates for larger females than for smaller females. In addition, the size of the first and second ejaculates transferred to a female by a male *H. crenulatus* were not significantly different, whereas the first was larger than the second for *H. sexdentatus*.

A parasitological survey was undertaken of the four grapsid crabs and the presence, seasonal variation and relationship with host gender and size of parasites determined. Four internal parasites were discovered: *Nectonema zealandica* n. sp. (Nematomorpha: Nectonematoidea), *Portunion* sp. (Isopoda: Entoniscidae), *Profilicollis novaezelandensis* n. sp. and *Profilicollis antarcticus* (Acanthocephala: Polymorphidae). *Portunion* sp. castrated its female hosts, but not the males thereby creating a more male-biased sex ratio. Males parasitised with *Portunion* sp. were equally successful during male-male competition and the number of matings they achieved.

The above findings are important for our current understanding of mating strategies in Grapsidae, which are more diverse than previously thought. Females with a restricted duration of sexual receptivity have some control over their receptive period and can therefore influence the OSR and the extent of male-male competition. As females mated multiple times during their receptive period, sperm competition is a common feature in Grapsidae. However, males employed different tactics in regards to sperm competition such as longer mating duration (e.g., *C. lavauxi*), high number of matings (*Helice crassa*), or post-copulatory mate guarding until oviposition (*Hemigrapsus* spp.).

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1 Introduction

Animal reproduction often involves complex behavioural processes that have evolved to maximise reproductive success under particular biotic and abiotic conditions. The behavioural strategies employed in courtship, mating and parental care are collectively known as 'mating systems' (Emlen & Oring 1977). A mating system can include such aspects as the location of mates, the number of mates acquired, the duration and characteristics of pair bonds, and patterns of parental care (Emlen & Oring 1977). Furthermore, the mating strategy of a species will be affected by the duration of sexual receptivity, i.e., the time available for mating, and the presence and outcome of sperm competition (defined as competition between ejaculates of two or more males for the fertilisation of the ova of a single female; Parker 1970).

Sexual selection arises from differences in reproductive success caused by competition for mates (Darwin 1871). Sexual selection is the driving force for differences among the sexes in size, shape, coloration and behaviour. In decapod Crustacea, for example, males are often larger and have larger chelipeds than females which has been shown to be important during male-male competition (e.g., Reid et al. 1994; Sainte-Marie et al. 1999). Furthermore, sexual selection is believed to be the cause of extravagant and conspicuous secondary sexual traits such as bright colours and huge feather plumes in many bird species that are otherwise not necessary for the survival of the individual. For example, male fiddler crabs, *Uca* spp., have one of their two chelipeds enlarged and use this for defence and threat during inter-male combat and for courtship, whereas females possess two small chelipeds (Christy & Salmon 1984).

The intensity of mate competition and sexual selection depends to a large degree on the operational sex ratio (OSR), which is the ratio of fertilisable females to sexually active males at any given time (Emlen & Oring 1977). The OSR varies with the degree of the spatial and temporal distribution of the limiting sex. Females are often the limiting sex as they are commonly receptive only temporarily. A strongly male-biased OSR should lead to stronger sexual selection for male traits favoured in competition over mates.

The reproductive success of a male will be higher if the male develops behavioural or morphological traits that increase the likelihood of the utilisation of his sperm (as opposed to that of another male) and therefore increases his paternity chances. Such traits could be mate-guarding, deposition of mating plugs, displacement of a competitor's sperm, and frequent or prolonged copulation (Birkhead & Parker 1997).

Sexual selection, female receptivity, and sperm competition have been the subjects of many studies over the last two decades (see review of Anderson 1994, Eberhard 1996, Birkhead

& Møller 1998), with emphasis on birds, insects, and fish. By comparison, little is known about these topics for marine invertebrates such as crustaceans.

A classification of mating associations of brachyuran crabs based on modes of competition among males for females was developed by Christy (1987). Competition among males was divided into female-centered competition (i.e., males search for or attract receptive females which they defend from other males), resource-centered competition (i.e., males defend resources that females require for breeding or survival), and encounter rate competition (i.e., males try to maximise the rate at which they encounter females, but neither defend females nor resources) (Christy 1987). Differences in patterns of predation, competitor density, density, distribution, and mobility of females, habitat requirements for breeding, and mate choice are factors influencing male strategies (Christy 1987). These modes of male competition are related to the spatial and temporal distribution of receptive females and are influenced by the differences in their habitat.

In addition, male strategies in relation to mate guarding of Crustacea have received particular attention in species that mate immediately following moulting (Wickler & Seibt 1981; Grafen & Ridley 1983; Ridley 1983; Jormalainen 1998; Bauer & Abdalla 2001). In many Crustacea mating and moulting are linked such that females are only receptive for a short time after moulting until the exoskeleton has hardened (i.e., postmoult mating) (Hartnoll 1969). In other Crustacea, including Grapsidae, mating occurs during the intermoult when the exoskeleton is hard (Hartnoll 1969). Postmoult mating is common in fully aquatic families which often use pheromones and tactile stimuli as their main means for mate recognition, whereas intermoult mating is more common in semi-terrestrial species which predominantly use auditory and visual stimuli for mate recognition (Hartnoll 1969; Salmon 1983). However, some species such as the lobster *Homarus americanus* (Waddy & Aiken 1990) and the tanner crab *Chionoecetes bairdi* (Donaldson & Adams 1989) mate after moulting and during intermoult.

Female choice in brachyuran crabs has been observed as resisting courtship and copulation attempts or choosing males on the basis of resource quality and courtship signals (Christy 1987). It is not known, however, if cryptic female choice occurs ("a female controlled process of structure that selectively favors paternity by conspecific males with a particular trait over that of others that lack the trait when the female has copulated with both types", Eberhard 1996) and how it influences the outcome of sperm competition.

Studies on aspects of mating systems of Crustacea have concentrated on Decapoda and included to a large degree commercially important species such as lobsters (e.g., Cowan & Atema 1990; Bushmann & Atema 1997; MacDiarmid & Butler 1999), shrimps (Bauer 1991;

Bauer 1996; Bauer & Abdalla 2001), and large edible crabs (e.g., Jivoff 1997 a,b; Jivoff & Hines 1998 a,b,; Sainte-Marie et al. 1997, 1999, 2000; Rondeau & Sainte-Marie 2001), or species that display some conspicuous reproductive behaviour such as the fiddler crabs (e.g., Christy & Salmon 1984, 1991; DeRivera & Vehrenkamp 2001; Murai et al. 2002). In comparison, Grapsidae have received little attention although they are a common and abundant family along most temperate and tropical shores and estuaries (e.g., Griffin 1971; Burggren & McMahon 1988; Fukui 1988). Recent studies relating to grapsid reproduction have been concerned with general reproductive biology (e.g., Seiple & Salmon 1982; Abele 1986; Flores & Negreiros-Fransozo 1998; McDermott 1998), mating behaviour (e.g., Abele et al. 1986; Fukui 1993, 1994, 1995), reproductive structures, and sperm storage (e.g., Anilkumar et al. 1996, 1999; López Greco et al. 1999).

The present study focuses on the mating strategies of four intertidal grapsid crab species common in New Zealand: the purple rock crab, *Hemigrapsus sexdentatus* (formerly known as *H. edwardsii*), the hairy handed crab, *H. crenulatus*, the tunnelling mud crab, *Helice crassa*, and the smooth shore crab *Cyclograpsus lavauxi*. The major objectives were to describe their mating behaviour and to investigate factors that influence the duration of female receptivity, male-male competition, and sperm competition, and to compare and discuss these in the context of mating strategies.

In particular, I explored whether the absence or presence of males affects the duration of sexual receptivity of female *Hemigrapsus sexdentatus* in the field (Chapter 2) and laboratory (Chapter 3). I examined whether laboratory conditions affect the onset of the breeding season of female *H. sexdentatus* (Chapter 2) and *H. crenulatus* (Chapter 4). I tested whether male size influences mating success and examined the extent that male-male and sperm competition occurs in these species. Furthermore, I was interested in whether males display behaviour that ensures paternity and reduces the risk of sperm competition (Chapters 3 and 4). The mating activity in the field of *H. sexdentatus* and *H. crenulatus* was estimated using information on spermatheca weights obtained from mating experiments in the laboratory (Chapters 3 and 4). I investigated whether the OSR influences the mating strategy or the fertilisation window of *Hemigrapsus crenulatus* (Chapter 4). In addition, I compared the mating behaviour of un-infected male *H. crenulatus* and males parasitised with *Portunion* sp. (Crustacea: Isopoda)

Similar to the studies on the two *Hemigrapsus* species, I examined mating behaviour of *C. lavauxi* and *Helice crassa* (Chapter 5). In particular, I was interested in the duration of female receptivity, mating frequency, whether male size is important for male mating success, and the effect of the parasite *Portunion* sp. on reproductive behaviour. In addition, I investigated

whether *Helice crassa*, a burrowing species, mates inside the burrows as well as on the surface. The main results on the mating systems known from other Grapsidae are reviewed in this chapter in a comparative analysis.

Initial observations revealed that the four grapsid crabs studied here were hosts to several parasite species, including some new to science (Appendices 10.1 and 10.2). Some achieved high parasitism rates of up to 34.1%. Parasites have been shown to reduce the mating success of their hosts (Morris et al. 1975; Read 1988; Clayton 1990; Milinski & Bakker 1990) and to substantially reduce reproduction of their host by castrating them. Host castration can be achieved by a parasite feeding directly on the gonads of the host or indirectly by diverting energy away from gonad development, or by influencing the host hormonal balance (Baudoin 1975; Coustau et al. 1991; Schallig et al. 1991). Furthermore, parasitic castrators can cause changes in secondary sexual characters as well as host viability and growth (Barnard 1990). Parasites are, therefore, able to exert considerable influence on mate competition and the operational sex ratio, particular when parasitism is high.

Parasites and the evolution of host sexual behaviour have received increased attention over the last twenty years (Milinski 2001). The Hamilton-Zuk (1982) hypothesis of parasite-mediated sexual selection suggests that genetic cycles in hosts and parasites maintain fitness heritability. It was hypothesised that the choosing sex favours healthy mates which are able to display costly traits such as bright red spots and therefore indicate better resistance to parasites. However, although several studies have supported important aspects of parasite-mediated sexual selection, the relative importance of parasites in shaping mating systems is still controversial (Hamilton & Zuk 1989; Read 1990; Anderson 1994).

Currently, there is only limited information available on aquatic parasites of New Zealand's marine fauna. Previous studies are mostly restricted to parasites and diseases of commercially important fish and shellfish species, whereas crustaceans received very little attention in New Zealand (Hine & Jones 1994), and no parasitological surveys have been carried out on New Zealand grapsid crabs previously.

A further objective of my study was to explore the parasite fauna of the four grapsid crabs (*C. lavauxi*, *Helice crassa*, *Hemigrapsus sexdentatus*, and *H. crenulatus*) and to determine the presence, seasonal variation and relationship of several internal parasites with host gender and size. In addition, I wanted to investigate the impact of the parasites on reproduction and mating behaviour.

Chapter 6 presents results about the ecology of the entoniscid parasite *Portunion* sp. (Crustacea: Isopoda) which was discovered in three crab species (*C. lavauxi*, *Helice crassa*, and

Hemigrapsus crenulatus) and its effect on their reproduction. The occurrence of the different developmental stages of *Portunion* sp. in the hosts are described. Chapter 7 discusses the prevalence of cystacanths of *Profilicollis* sp. (Acanthocephala: Polymorphidae) in relation to the two intermediate hosts *Helice crassa* and *Hemigrapsus crenulatus* as well as to the presence of possible definitive hosts. Furthermore, the morphological features of cystacanths of the genus *Profilicollis* are compared to provide an overview of these developmental stages, which are usually ignored in comprehensive identification keys of adult descriptions.

The first part of this thesis therefore focuses on the mating strategies of four grapsid crabs and includes observations on mating behaviour and factors influencing female receptivity, male-male competition and sperm competition (Chapters 2 – 5), as well as a comparison of the mating behaviour of unparasitised crabs and crabs parasitised with the unusual internal parasite *Portunion* sp. The second part describes host-parasite associations with comments on the effects on reproduction (Chapters 6 and 7). In the Appendix (10.1 and 10.2), I have included the publications of the descriptions of the two new parasites *Nectonema zealandica* (Nematomorpha: Nectonematoidea) and *Profilicollis novaezelandensis* (Acanthocephala: Polymorphidae) and information on the parasites distribution in their hosts.

The family Grapsidae is currently undergoing a systematic revision that may possibly result in the establishment of several families, e.g., Gecarcinidae, Glyptograpsidae, Grapsidae, Plagusiidae, Sesarmidae, and Varunidae (Schubart et al. 2000; Schubart et al. 2002). Based on recent taxonomic classification, *Cyclograpsus lavauxi*, *Hemigrapsus crenulatus* and *H. sexdentatus* are now included in the family Varunidae (Cuesta et al. 2001) and *Helice* is likely to be included in the same family. As this revision is still in progress, I refer to the family Grapsidae here in the broader sense, i.e. *sensu lato*, which includes all species formerly included in the family Grapsidae.

Footnote:
Chapters 2 to 7 have been prepared in such a way that they are independent from each other and with submission for publication in mind. Therefore, some overlap in the description of the methods and background information does occasionally occur in these chapters.

2 Factors influencing the receptivity of female purple rock crabs, *Hemigrapsus sexdentatus* (Brachyura: Grapsidae)

Abstract - Field and laboratory observations were carried out in New Zealand to investigate factors influencing the receptivity of female purple rock crabs, *Hemigrapsus sexdentatus*. The onset and duration of female receptivity is of interest because it influences the time available for mating, the operational sex ratio and it could have an effect on male-male competition and the extent of sperm competition. Females were receptive once a year for a short time prior to oviposition. The breeding seasons over the duration of three years were highly synchronised and lasted for about three weeks from the end of March to mid-April (southern hemisphere autumn), by which time almost all mature females had laid eggs. In the field, few receptive females were found during the breeding season (0% to 4.9%), even though a large number of females were examined (935 in 1999, 555 in 2000). This suggests that in the field females are receptive for less than a day, otherwise more receptive females should have been found during the short breeding season. The onset of the breeding season was the same for the wild population and crabs collected from the same location and held in field cages, however, the duration of receptivity increased to several days for caged females. The duration of receptivity of isolated females increased significantly with size. Females isolated from males stayed receptive significantly longer (5.5 days) than females caged with males (3.3 days) in field experiments. This suggests that females have some control over the duration of their receptivity and the time available for mating prior to oviposition, and that they can influence receptivity according to the presence or absence of males. Based on my results and results of other studies on female receptivity, it appears that some Crustacea from both mating systems (postmoult and intermoult), have control over the onset or duration of their receptivity. Therefore, receptivity is not, as previously thought, solely dependent on internal factors, such as the developmental stage of the ovaries, the moult cycle or on environmental factors, but appears to be under partial female control. This is an important finding as female receptivity will have an impact on male-male and sperm competition and will therefore influence the outcome of sexual selection.

2.1 Introduction

Sexual selection, sperm competition and female choice have been the subjects of many studies over the last two decades (see reviews of Anderson 1994; Eberhard 1996; Birkhead & Møller 1998), especially on birds and insects. By comparison, little is known on these subjects for marine invertebrates such as crustaceans. In particular, it is uncertain whether females have any control over the duration of their receptive period and the fertilisation of their ova. More importantly, there has been no way to determine female receptivity, other than by inducing mating. Studies have been carried out on the general mating behaviour, mate choice, male-male competition and sperm storage for marine crustaceans (Christy 1987; Diesel 1991; Subramoniam 1993), but this did not reveal anything about the factors influencing female receptivity. The time and duration of female receptivity is important as it will determine the operational sex ratio (OSR, defined as the ratio of fertilisable females to sexually mature males at any given time; Emlen & Oring 1977), and the extent of male-male and sperm competition during this time. For example, it has been shown that the OSR influences female preference and male-male competition in guppies (Jirotkul 1999). Females of the hermit crabs *Pagurus filholi* and *P. lanuginosus* were guarded earlier and longer by males when the sex ratio was male biased (Wada et al. 1999; Minouchi & Goshima 2000). Females of the white-tailed moth, *Elcysma westwoodii*, choose males by limiting their own receptivity when males occur in high densities and hence employ a mechanism of 'passive female choice' (Koshio 1996). In contrast to the self-control of female receptivity, it has been shown for the planthopper, *Prokelisia dolus*, that a substance in male ejaculates inhibits female sexual receptivity (Heady 1993). A decrease or absence of sexual receptivity of mated females has also been shown for several insects and spiders, although the mechanisms for this reduction are often not fully understood (see Ringo 1996; Elgar 1998).

So far it has been mostly assumed that female crustaceans cannot control their receptivity. It was argued that the onset and duration of female receptivity were most likely dependant on either some internal factors such as the stage of moulting or development stage of the ovaries, which are often regulated by hormones (DeKleijn & VanHerp 1998), or external environmental factors such as lunar cycle, temperature, or photo-period (Flores & Negreiros-Fransozo 1998), or a combination of both (Caubet et al. 1998). For example, the percentage of receptive females of the ocypodid crab *Ilyoplax pusilla* peaked near full and new moons (Henmi & Murai 1999); the breeding activities of *Orconectes rusticus* were initiated by the rising water temperatures in spring (Berril & Arsenault 1982); females of the intertidal amphipod *Corophium volutator* moulted synchronously during spring tides, after which they were receptive for a few days

(McCurdy et al. 2000); receptive females of *Uca pugnax* occurred in a semi-monthly cycle and it was suggested that receptivity was influenced by some underlying neural and hormonal cycle (Greenspan 1982); and, receptivity of female *Sesarma* sp. was also suggested to be under lunar-influenced hormonal control (Zimmerman & Felder 1991).

In many Crustacea mating and moulting are linked such that females are only receptive for a short time after moulting until the exoskeleton has hardened (i.e., postmoult mating). In these cases, the onset and duration of female receptivity (time available for mating) is determined by the moult cycle and often limited to several hours for *Cancer gracilis* (Orensanz et al. 1995), 5 - 12 hours for the portunid crab *Callinectes sapidus* (Gleeson 1991), and 6 - 50 hours for the marine isopod *Paracerceis sculpta* (Shuster 1989). However, receptivity may last for up to 21 days for the tanner crab *Chionoecetes bairdi* (Donaldson & Adams 1989). In contrast, other Crustacea mate during the intermoult, when the exoskeleton is hard, commonly prior to oviposition. The time and duration of female receptivity in these cases is usually not known. However, from the few examples that are known, it appears that females are receptive anywhere from a few days up to several weeks and may include the days before and after oviposition. For example, females of grapsid crabs are receptive for 1-2 days (*Gaetice depressus*, Fukui 1993) or 2-3 days (*Sesarma* sp. (*reticulatum*), Zimmerman & Felder 1991), and females of ocypodid crabs for an average of 11.4 days (*Macrophthalmus hirtipes*, Jennings et al. 2000) or up to two weeks (*Ilyoplax pusilla*, Henmi & Murai 1999). Furthermore, some species are able to do both: mate whilst soft-shelled after moulting and subsequently when hard-shelled, as found in the tanner crab *C. bairdi* (Donaldson & Adams 1989) and the American lobster, *Homarus americanus* (Waddy & Aiken 1990). Females of other species are assumed to be continuously receptive to mating after reaching a certain developmental stage. For example, females are receptive continuously in the marine isopod *Jaera hopeana* from the manca-II stage onwards (Franke 1993) and after developing permanently mobile gonopore opercula in the case of the fiddler crab *Uca vocans* (Salmon 1984), and in the grapsid crab *Pachygrapsus transversus* (Abele et al. 1986). For *U. vocans*, however, a peak of mating activities was observed during spring tides (Salmon 1984). Overall, when both systems (postmoult and intermoult mating) are compared, it appears that intermoult mating allows more flexible timing of mating in relation to biotic and abiotic variables than the more physiologically scheduled behaviours associated with postmoult mating.

The intertidal crab *Hemigrapsus sexdentatus* (formerly known as *H. edwardsii*) is endemic to New Zealand, where it occurs on relatively sheltered rocky or stony shores (McLay 1988). Female *H. sexdentatus* have gonopores that are covered by an opercula. For most of the year,

these gonopore opercula are calcified and immobile, which inhibits any mating activities. In laboratory observations, I found that the gonopore opercula of *H. sexdentatus* become mobile during the intermoult a few days before oviposition. Females then become immediately receptive and attractive to males (personal observation). During the receptive period, females can mate multiple times with the same or different males. Males guard females after mating until oviposition by covering and holding them tightly to avoid subsequent matings by other males, which would cause sperm competition. Despite this guarding, females often mate with several males as larger males can displace smaller ones. Although female gonopore opercula remain mobile for several days after oviposition, no attempts were made by males to mate with ovigerous females. Females of this species are able to store sperm from the previous mating season and can fertilise their ova without re-mating the following year (personal observation).

In this study, I examined the breeding season of the purple rock crab *H. sexdentatus* with particular emphasis on factors influencing the onset and duration of female receptivity prior to oviposition, under field and laboratory conditions. A population in the field was monitored to obtain information on the spatial distribution of crabs, especially receptive females, their movement and density before, during and after the breeding season. The duration of the receptivity of individual females was examined in the presence and absence of males in field cages. In addition, a group of females was observed in the laboratory to compare female receptivity under laboratory and field conditions. This study was therefore aimed at determining the duration of the fertilisation window of females of *H. sexdentatus* and under which conditions the duration of this window changes.

2.2 Materials and Methods

Observations and collections of *H. sexdentatus* were made at an intertidal boulder field (approximately 30 m wide x 56 m long) of a sandy beach north of Christchurch, South Island, New Zealand (43° 06′ S, 172° 53′ E) from 1998 to 2000. In addition, a number of crabs (see details below) were held and observed in the laboratory, under a 12 h light: 12 h dark cycle in tanks with circulating seawater of 12 - 15°C.

Breeding season, movement and density in the field with emphasis on the occurrence of receptive females

To examine the breeding season and occurrence of receptive females in their natural habitat, observations of a population in the field were carried out using several methods which included mark-and-recapture sampling, transect and random sampling.

Female receptivity was determined by noting whether the gonopore operculum was mobile or immobile. To determine operculum mobility, the abdomen was slightly lifted and one of the two opercula was probed carefully with fine forceps. When the opercula were mobile and could be pushed inwards like a trapdoor, females were considered receptive. If the opercula were immobile and could not be moved by light pressure, females were classified as non-receptive. Ovigerous females, had mobile opercula up to four days after oviposition (laboratory observations), but were assumed to be non-receptive, as matings were never observed in the laboratory once a female had laid eggs.

To determine the occurrence of receptive and ovigerous females during the year, monthly samples were taken in 1998. Crabs were collected randomly by hand from the entire boulder field. Females were checked whether they were receptive or carried eggs. A total of 1844 crabs (1125 females and 719 males) were examined in the middle of each month (January: 111 females / 71 males, February: 82/66, March: 101/77, April: 87/40, May: 144/25, June: 97/41, July: 107/ 47, August: 54/61, September: 106/80, October: 103/73, November: 45/59, December: 88/79, respectively). The majority (95.2%) of the crabs collected were mature (≥ 24 mm CW).

To obtain information on movement of crabs in the field, in particular receptive females, a mark-and-recapture experiment was set up in which crabs were marked, released and then searched for at specific locations.

The field site was searched intensively for crabs during low tide on 16 February 1999, which was about 6 weeks before the start of the breeding season. All crabs found were measured (carapace width (CW), using a Mitutoyo digital callipers to the nearest 0.1 mm) and sexed (using the relative abdomen width; females have a wider/broader abdomen than males). Mature crabs (≥ 24 mm CW) were individually tagged with small, coloured, numbered bee tags (round plastic discs of 3 mm diameter glued to the carapace with cyanoacrynalate glue), as long as no more than four pereopods were missing. About half of the mature crabs were tagged at the site and returned evenly over the entire boulder field the same day at low tide, the other half was tagged in the laboratory and returned the next day at low tide. Figures 2.1 and 2.2 show details of sizes and numbers of crabs collected and tagged as well as the size distribution of males and females in the population. A few of the males and females, between 24 mm and 32 mm CW, were kept

in the laboratory for observations of reproductive behaviour. In addition, at the end of March, 21 unmarked females were collected from the site and brought to the laboratory. These females were checked daily for the onset of receptivity, which would indicate the onset of the breeding season in the field.

Fifteen rocks in the boulder field were marked with large numbers using fluorescent paint spray between 9 and 29 March 1999. These rocks (about 30 cm wide and 40 cm long) were located over the entire boulder field and had one or more tagged crabs underneath at the time of selection. The marked rocks were lifted up carefully and checked underneath for crabs once the mating season started. Crabs from each rock were collected in a bucket, checked, and then returned to the same spot within a few minutes. Until 31 March the area underneath the rocks and a small area around the rocks were searched, but only small numbers of crabs were relocated. The search area was then extended including a diameter of about 1.5 m around the rock in order to obtain larger samples. The following records were taken for crabs found under each rock: number of mature crabs, sex, tag number, condition of the female (receptive or not, presence or absence of eggs) and number and size of paired crabs (males guarding receptive females by holding one of her walking legs with his chelipeds and/or covering her between his legs). Observations were carried out at low tide during daytime hours, except for 21 and 31 March and 2 April, when it was done at night using a torch (see results for details on other collection dates). These night observations and collections were carried out in the expectation that free moving crabs would be easily observed. As this was not the case, collections were mainly done during daytime hours.

To obtain additional information on the occurrence of pairs, the entire boulder field was searched thoroughly in March and April 1999 (once per week before the mating season and about every third day during the mating season).

As I found very few receptive females in the 1999 breeding season (see results for details), I wanted to explore the possibility of crabs leaving the study area to mate somewhere else, for example sub-tidally. To determine whether the density of mature crabs in the observation area changes over time, as well as to examine the occurrence of receptive and ovigerous females, collections were carried out before, during and after the breeding season in March, April and May 2000. Mature crabs (≥ 24 mm CW) were collected by hand along a 40 m transect using 1 m x 1 m squares every 4 m, beginning at the highest point of the boulder field down towards the water line. Four transects were taken weekly from 11 March until 9 April (i.e., 8 transects before and 12 transects during the breeding season) and four transects on 22 May (i.e., 4 after the breeding season). Notes were taken on crab numbers, size (CW), sex, mobility of female

gonopore opercula, the presence or absence of eggs and male-female pairs (i.e., male guarding female, see also above). In addition, five samples (one on each of the following days: 23, 28, and 31 March and 7 and 13 April) were taken randomly across the boulder field to obtain additional information on the percentage of ovigerous females. The sex of the crabs and whether or not the females were carrying eggs were noted for these random collections. Crabs were returned to the same place immediately after examination.

Effects of the presence and absence of males on female receptivity

To examine the onset and duration of receptivity of individual females in the presence and absence of males in their natural environment, mature crabs were placed in field cages and observed during the breeding season. Cages (25 cm x 25 cm wide, 9 cm high, plastic-coated 18 mm wire mesh) were placed between boulders to prevent them from being washed away and contained two rocks to use as shelter by the crabs. These cages prevented crabs from escaping and the mesh size was small enough to prevent mating through the mesh with crabs outside the cage. In March 2000, a total of 51 field cages were placed in the field, of which 24 housed two females and no males, and 27 housed a female together with a male. Most of the cages (16 cages with two females and 17 cages with one female and one male) were placed in the field on 11 March, a week before the breeding season in the field started. The remainder of the cages (8 cages with two females and 10 cages with one female and one male) were placed in the field during the breeding season on 29 March. Females were checked daily during the breeding season for their receptivity and the presence of eggs until mid April, which was about the end of the breeding season. Crabs held in cages were fed opened blue mussels (*Mytilus edulis*) two to three times a week. Crabs were removed from the cages in mid April. Females from cages with attending males, were taken to the laboratory to examine the spermatheca contents. Full spermatheca would indicate that they actually mated with the male in the cage during the breeding season. Crabs were killed by placing them in a freezer at -15°C for about 1 hour. After removal of the carapace and some internal organs, the spermatheca were examined and dissected out by a cut close to the gonopore. A spermatheca was considered full, when they were clearly visible as two large, round, fully filled 'balloons' as soon as the overlying internal organs were removed, and filled with white ejaculates.

Onset and duration of the breeding season under laboratory conditions

To examine the onset and duration of female receptivity under laboratory conditions, mature female crabs were collected randomly by hand from the entire boulder field on 15 January, 15

February, 15 March, and 18 December 1998, and 18 February and 10, 21 March 1999, and taken to the laboratory. Crabs were held in tanks and fed blue mussels two to three times a week. To assess female receptivity the gonopore opercula were probed weekly until the end of February and daily in March and April in both years (which equals the time before and during the breeding season) until all females became receptive.

Terminology and Statistics

The sex ratio was calculated by dividing the number of mature females by the number of mature males. The operational sex ratio was calculated by dividing the number of receptive females by the number of mature males. Females of *Hemigrapsus sexdentatus* are referred to as being receptive, when they have mobile gonopore opercula prior to oviposition. The duration of female receptivity was defined as the time from the first day a female had mobile gonopore opercula up to the day she laid eggs. Data were analysed using SYSTAT 9. Percentages were arcsine transformed before statistical analyses were carried out. Mean values given are followed by the standard error of the mean.

2.3 Results

Breeding season in the field

Ovigerous female *H. sexdentatus* were present in the field from April (95.3% females ovigerous) to July (73.8% females ovigerous) in samples taken mid-monthly in 1998 (Fig. 2.3). Females laid eggs once per year and carried them for about 3.5 months (according to observation on egg development, data not shown). The onset of the breeding season was highly synchronised every year. In 1999 and 2000, the first ovigerous females appeared at the end of March and within two to three weeks the majority of females had laid eggs (Fig. 2.4). Females collected mid-March in 1998 and held in the laboratory also started to become receptive at the end of March and by mid April, 95.3% of the females were ovigerous.

Receptive females were extremely rare in the field at any one time (maximum of 4.4% in 1999 and 4.9% in 2000) (Fig. 2.5). Only one receptive female was found out of 87 on 13 April 1998, 11 receptive females were found out of 935 mature females examined from 21 March to 21 April in 1999, and 12 out of 555 examined from 11 March to 9 April in 2000. Except in one case, receptive females were always guarded by a male, which was sitting on top of the female, caging her underneath with his chelipeds and legs and often holding a female pereopod with one

of his chelipeds. Receptive females were therefore easily recognised in the field, even before probing the opercula for their mobility.

The sex ratio of the mature crabs in March and April was mostly female-biased and ranged from 1.0 to 2.0 females per male in 1999 and from 1.0 to 2.5 in 2000 (Fig. 2.6). In contrast, the operational sex ratio was always highly male-biased ranging from 0.027 to 0.074 receptive females per male in 1999 (equivalent to 14 to 37 males per receptive female), and from 0.029 to 0.068 receptive females per male in 2000 (equivalent to 15 to 34 males per receptive female) (Fig. 2.6).

Density and movement in the field

The mean density of mature crabs varied between 1.3 and 2.0 males per m^2, and between 1.9 and 3.3 females per m^2 from March to May 2000 (Fig. 2.7). The densities of males and females before, during and after the mating season were not significantly different (ANOVA, males: $F_{2,21} = 2.1$, $P = 0.153$, females: $F_{2,21} = 3.0$, $P = 0.074$). This shows that crabs were not leaving the intertidal observation area for an extended period of time to mate elsewhere.

In the mark and recapture experiment during the 1999 breeding season, a total of 1579 crabs were examined within four weeks (21 March to 21 April), of which 14.2% were tagged crabs (112 males, 94 females). During this time, the recapture rate varied between 5.0% and 22.7% for males and between 3.6% and 15.9% for females, but did not change over time. The mean recapture rate was 18.1% for males and 11.8% for females (Fig. 2.8). The majority of marked crabs were recaptured only once. However, nine of the marked males and twelve of the marked females were found twice, and two of the marked females were found three times. Crabs that had been recaptured more than once allowed me to follow their movement over the boulder field during the breeding season. Crabs had moved either from the higher shore seawards (n = 9) or vice versa (n = 8). Therefore, there was no obvious trend in the directions of movement. One female was found under the same rock twice on consecutive sampling days. Several other crabs were also found twice under the same rock, but not on consecutive sampling days (1 male, 2 females). It appears therefore, that both male and female crabs are highly mobile at the study site.

Most of the marked females, that had been captured more than once, had either changed the mobility of the gonopore opercula and/or were carrying freshly laid eggs. For example, one female was recaptured first when she was non-receptive with immobile gonopore opercula and no eggs and nine days later she was collected again carrying eggs. The opercula of this female were still mobile, indicating that she just recently laid eggs. Non-receptive females were

recaptured as ovigerous females nine to 21 days later, however, it could not be determined how long these females were receptive before oviposition. In one case, a receptive female was captured. This female was recaptured three days later, carrying eggs and having mobile opercula. Seven days later this ovigerous female was again recaptured, but then with immobile opercula (Table 1). The exact period of receptivity could not be determined for this female. Overall, I know for several individually marked females the week they became receptive and laid eggs, and that the whole process can take less then nine days (see Table 1). As the rate of encountering receptive females was very low, I was not able to measure the receptive duration of individual females on a smaller time frame, i.e., in hours or fewer days. However, I concluded that females became receptive and laid eggs in the observation area.

Effects of the presence and absence of males on female receptivity

Females that had been placed in cages before the breeding season started to become receptive on 24 March 2000 and within 16 days, 98.0% of females had become receptive. There was no difference in the onset of receptivity in the presence or absence of a male (Fig. 2.9). Females stayed receptive for several days. The duration of receptivity of isolated females increased significantly with female size (linear regression, $R^2 = 0.137$, $P = 0.031$, N = 34) and a weak correlation was found for females housed with males (linear regression, $R^2 = 0.113$, $P = 0.117$, N = 23) (Fig. 2.10). In addition, females isolated from males stayed receptive significantly longer before oviposition (mean: 5.5 ± 0.3 days, N = 34) compared to females which were housed together with a male (mean: 3.3 ± 0.2 days, N = 23) (ANCOVA: $R^2 = 0.43$, $F_{1,54} = 36.80$, $P < 0.001$; carapace width covariate) (Fig. 2.11). During the course of the breeding season, ovigerous females were therefore first observed and more common in cages where males were present compared to the cages were males were absent (Fig. 2.12). This led to significant differences in the percentages of ovigerous females in the cages, where males were either present or absent, over the course of the breeding season (Paired t test: $t_{15} = 6.311$, $P < 0.001$). However, near the end of the breeding season on 13 April, most of the females that were placed in cages before the breeding season, had laid eggs whether (100%) or not (87.5%) a male was present (Fig. 2.12). Isolated females must have used sperm stored from the previous breeding season as they laid fertile eggs.

About half of the females that were placed in field cages during the breeding season had also laid eggs by the end of the observation period (46% females ovigerous). The remaining females were either still not receptive (2 of the 16 females in the cages with two females, and 2

of the 10 females held with a male) or receptive (8 of the 16 females in the cages with two females, and 2 of the 10 females held with a male).

Overall, the duration of the breeding season of females in cages and of the females in the surrounding 'free' population was similar. However, ovigerous females occurred about a week earlier in the surrounding population compared to the females in the cages (Fig. 2.12), most likely due to differences in the duration of female receptivity (see discussion). Therefore, significant differences were found for the percentages of ovigerous females in the field compared to females held with males in cages (Paired t test: $t_{10} = 2.758$, $P = 0.020$) and compared to females held without males in cages (Paired t test: $t_{10} = 9.536$, $P < 0.001$).

All females that had become receptive, and were held in cages together with males, had full spermatheca at the end of the experiment.

Onset and duration of the breeding season under laboratory conditions

Females were collected from one to four months before the breeding season and kept under laboratory conditions, where they were examined for their receptivity during the breeding season. Females that were collected first became receptive earlier than females collected later and females left in the field. In other words, the longer they were kept under laboratory conditions before the breeding season the earlier they became receptive (started the breeding season), and in addition, the longer the period of all females becoming receptive (Fig. 2.13). In 1998, for example, females collected in January, February and March started the breeding season on 11 March, 18 March and 26 March, respectively, and the period during which all females became receptive (laboratory breeding season) was 32 days, 26 days and 25 days, respectively.

2.4 Discussion

Breeding season and density in the field

Hemigrapsus sexdentatus is an autumn/winter breeder with one brood and showed a distinct annual cycle which has also been reported for *Hemigrapsus nudus* at the West Coast of North America (Boolootian et al. 1959; Knudsen 1964). Ninety-eight percent of *H. nudus* females became ovigerous within 3 months, albeit the majority of females (70%) laid eggs within the first 3 weeks of the breeding season. In contrast, *H. sexdentatus* has a much shorter and even more synchronised breeding season of about 2 to 3 weeks. In such a system, one would expect to find many receptive females at any time during the short breeding season. However, in our

case receptive females were very rare in the natural population during the breeding season, even though relatively many receptive females were present in our field cages. The pattern found in the field could be theoretically due to the fact that: a) females move temporarily out of the observation area (i.e., to sub-tidal areas) once they are or to become receptive and return to the shore when they are ovigerous, b) not all females mate, but directly lay eggs once they have mobile gonopore opercula, or c) females are receptive for a very short time (less than 24 hours).

The first scenario is not likely, as the density of *H. sexdentatus* did not change significantly before, during and after the breeding season. In addition, receptive females were present at the study site, guarded by males, and used the study area as a mating ground prior to oviposition. The second possibility is also not likely, as one of our earlier studies revealed that all ovigerous females in the field had freshly, fully-filled spermatheca and had therefore recently mated prior to oviposition (see Chapter 3). To investigate the third scenario further, I compared the occurrence of ovigerous females in the field with the cumulative percentage of newly receptive females held in the cages (Fig. 2.14). The latter presents the proportion of females that became receptive up to a particular date, which would also equal a pattern in which each female in the cage becomes receptive and ovigerous within a day. Figure 15 shows that the timing of the breeding season of both groups (uncaged and caged females) would then be almost identical and no significant differences occur between the percentage of ovigerous females in the field and the accumulative percentage of newly receptive females in cages (Paired t test: $t_{10} = 0.944$, $P = 0.368$). Assuming that caged females follow the natural pattern of the onset of the breeding season, it appears therefore that uncaged females are receptive for a shorter period of time compared to caged females (less than a day compared to two to eight days, respectively). A similar short receptive period of 1-3 and 2-3 days has also been reported for other grapsid crabs observed in the laboratory (Zimmerman & Felder 1991; Fukui 1993).

Onset and duration of the breeding season under laboratory conditions

Females held in the laboratory over an extended period of time started to become receptive earlier and had a longer breeding season compared to females in the field and females held in the laboratory for only a few days prior to the onset of the breeding season in the field. It has been shown previously that laboratory conditions have an affect on crustacean behaviour and physiology, such as changes in activity rhythms (Williams 1969), time of moulting (Zimmerman & Felder 1991), and reproductive events (Zimmerman & Felder 1991). Furthermore, spawning can be induced by changes in temperature and photoperiod in the shrimp, *Palaemonetes pugio* (Little 1968) and by changes in temperature in the blue crab *Callinectes sapidus* (Sulkin et al.

1976). My results also show clearly that one cannot determine the exact time of the mating season in the field from mating observations taken in the laboratory, although an indication of the time of mating in the field can be obtained.

It is not established yet, what exactly triggers the onset and the duration of the breeding season and therefore I can only speculate on the possible reasons for these differences between natural and laboratory conditions. Factors such as differences in water temperature, photoperiod, diet, or tidal activities may be important. It had been suggested that mating is triggered by a short photo-period for *Hemigrapsus nudus* (i.e., start at about the shortest day of the year) and by an increase in water temperature for *H. oregonensis* (Knudsen 1964) and *H. penicillatus* (Pillay & Ono 1978). As *H. sexdentatus* is an autumn/winter breeder its reproductive season commences when temperatures are falling and day-length is shortening.

In the present study, the water temperature was slightly colder (12-15°C compared to 16-17°C in the field) and day-length was shorter (12 hours compared to 13 hours at the beginning of March) in the laboratory than in the field, and this could have triggered breeding activities earlier in the season. Furthermore, crabs in the laboratory were not exposed to tidal changes and were fed on blue mussels, whereas in the field crabs inhabit tidal areas and have a more diverse food range. In addition, females only become receptive when they have ripe ovaries and factors influencing gonad development, will most likely have some impact on the general onset of the breeding season for this species.

Effects of the presence and absence of males on female receptivity

The onset of the receptive period of female *H. sexdentatus* was independent of the presence or absence of males. However, isolated females stayed receptive significantly longer and delayed oviposition for several days compared to females housed with males. Our results indicate therefore that females can partially control the duration of their receptivity. By contrast, in the grapsid crab *Gaetice depressus*, the period of receptivity for females was found to be similar whether males were temporarily present or absent for (Fukui 1993).

So far it has been mostly assumed that crustacean females cannot control their receptivity and that receptivity most likely depended on some internal or external factors. However, a few studies have begun to show a new pattern. For example, in the absence of males, females of the isopods *Paracerceis sculpta* and *Porcellio dilatatus* and the amphipod *Gammarus pulex*, appear to be able to delay initiation of their reproductive moult, after which they are receptive (Mocquard et al. 1976; Ward 1984; Shuster 1989). Females of *P. sculpta* and *G. pulex* moult eventually, but are either incapable of reabsorbing the un-inseminated ova and die without

reproducing (*P. sculpta*) or reabsorb the ova and recycle the material (*G. pulex*) (Ward 1984; Shuster 1989). Furthermore, females of the lobster *Homarus americanus* can stagger their moults to mate with a dominant male (Cowan & Atema 1990), and if females moult in an area where males are scarce and do not mate within 48 hours of moulting, they may remain receptive for up to 80 days (Snyder et al. 1992). Females of the caridean shrimp *Palaemonetes pugio* that mated after moulting, spawned within 2-3 hours after moulting, whereas isolated females could delay spawning for up to 2 days after moulting (Bauer & Abdalla 2001).

These examples demonstrate that in both crustacean mating systems (postmoult and intermoult) some females can exercise influence over the onset or the duration of their receptivity and control it according to the presence or absence of males. These females can therefore influence the operational sex ratio, which in turn will influence the intensity of male-male and sperm competition. Females could then benefit from this competition and mate with a high quality male. In addition to the 'fine-scale' adjustments by the female, other endogenous or exogenous factors could also influence the general timing of the breeding season. The mechanisms responsible remain to be established in detail.

In general, species with a synchronous breeding season will have a less male biased operational sex ratio (OSR), as many receptive females are available at the same time, compared to an asynchronous breeding population, where the OSR is highly male biased, because females are in short supply. Females of synchronised breeders could counteract this pattern in having a very short receptive period, which will increase the OSR, lead to stronger sexual selection and therefore to an increase of male-male and sperm competition. Females of *H. sexdentatus* fall in this category, as they appear to have a very short receptive period. However, they retain the possibility of extending the receptive period to increase the chance of mating if males are absent or in short supply (even though they can fertilise their ova with sperm stored from the previous year).

Table 2.1 Reproductive stages (non-receptive, receptive, ovigerous with mobile and immobile opercula) of individually tagged females of *Hemigrapsus sexdentatus* and their changes between recapture dates in 1999.

recaptured females (n)	non-receptive female	receptive female	ovigerous female with mobile opercula	ovigerous female with immobile opercula	days between observations
1	+		+		9
4	+			+	10, 14, 18, 21
1			+	+	9
3	++				3, 3, 3
3				++	4, 9, 16
1	+		+	+	10 - 8
1		+	+	+	3 - 7

+, indicates reproductive stage at which female was found

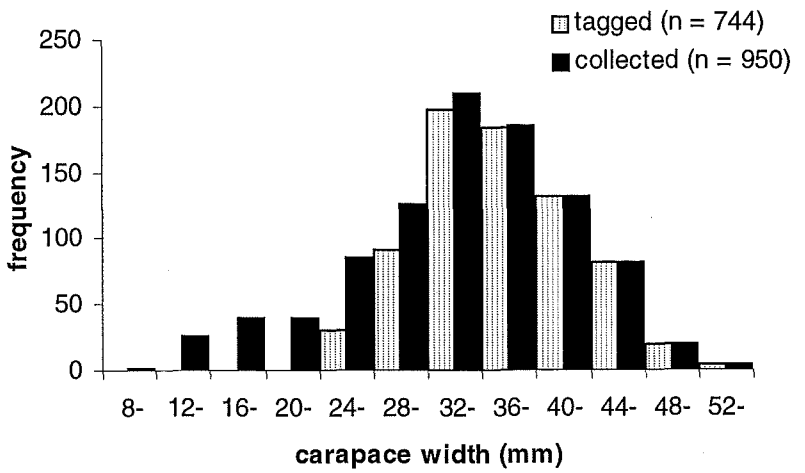


Figure 2.1 Size frequency distribution of *Hemigrapsus sexdentatus* collected and tagged in February 1999 (males and females combined).

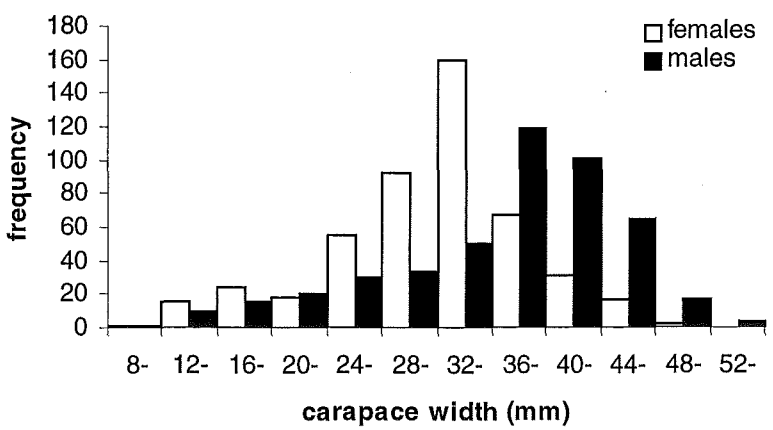


Figure 2.2 Size frequency distribution of males and females of *Hemigrapsus sexdentatus* collected in February 1999.

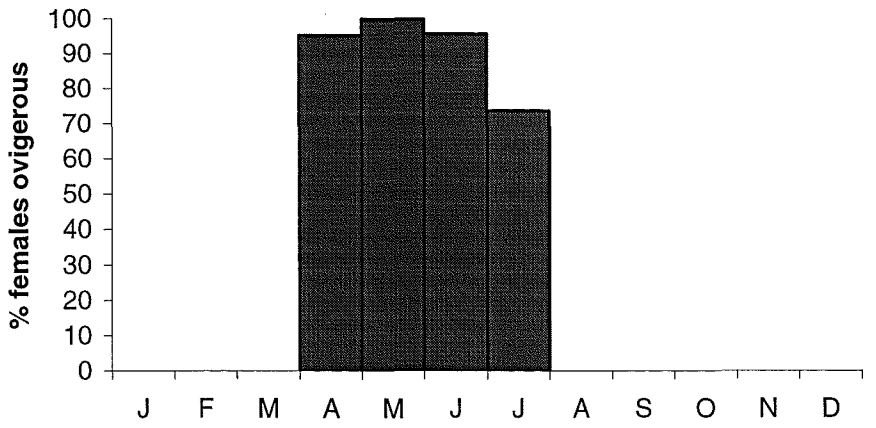


Figure 2.3 Percentage of ovigerous females of *Hemigrapsus sexdentatus* in monthly samples collected from January to December 1998.

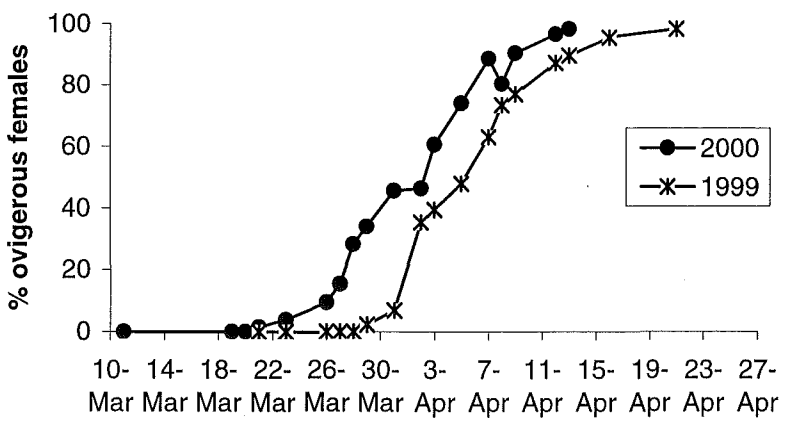


Figure 2.4 Percentage of ovigerous females during the breeding seasons of *Hemigrapsus sexdentatus* in 1999 and 2000 in Canterbury, New Zealand. Data for ovigerous females in 1999 were obtained from mark and recapture experiments (935 females examined), and in 2000 from transect and random sampling (870 females examined).

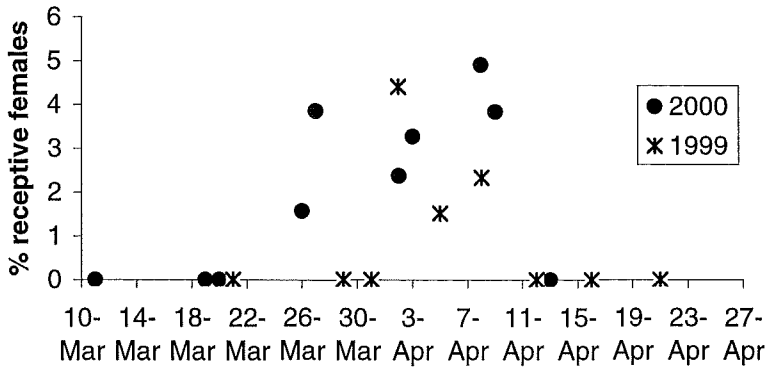


Figure 2.5 Percentage of receptive mature females of *Hemigrapsus sexdentatus* in 1999 and 2000. Data for receptive females in 1999 were obtained from mark and recapture experiments (935 females examined), data in 2000 were obtained from transect sampling (555 females examined).

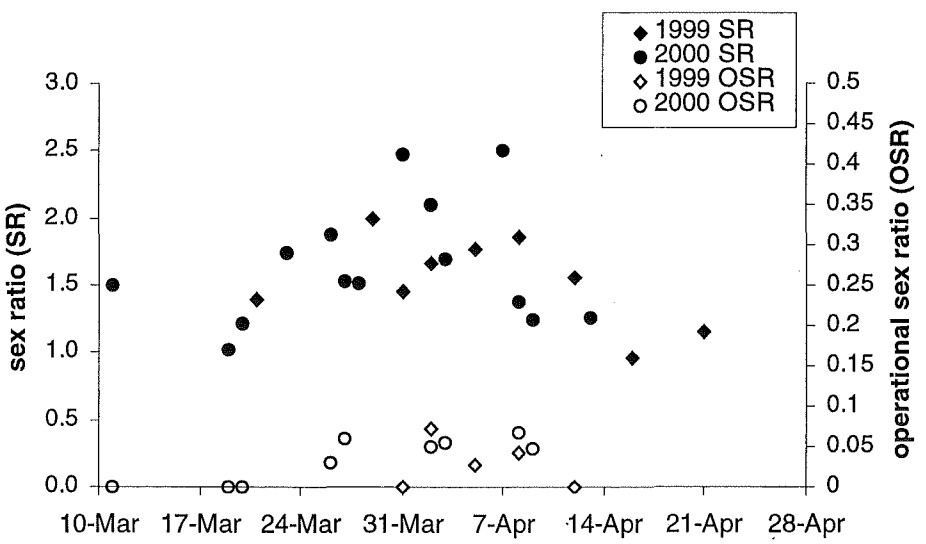


Figure 2.6 Sex ratio (SR, females per male, filled symbols) and operational sex ratio (OSR, receptive females per male, open symbols) of *Hemigrapsus sexdentatus* in March and April. Data were obtained from mark and recapture experiment in 1999 (644 males, 935 females), and from transect and random sampling in 2000 (548 males, 870 females).

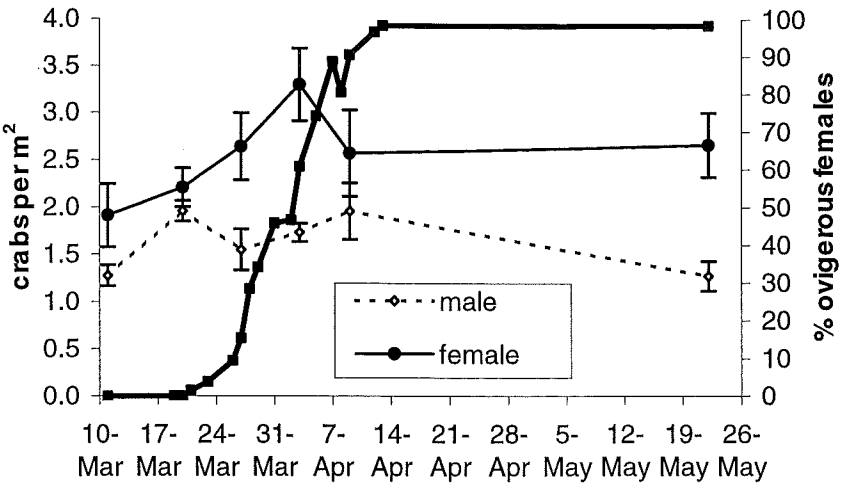


Figure 2.7 Density of *Hemigrapsus sexdentatus* before (11/3, 20/3), during (27/3, 3/4 , 9/4), and after (22/5) the breeding season and the percentage of ovigerous mature females from March to May 2000. Density data were obtained by transect sampling (428 males, 611 females). Data for ovigerous females were obtained from transect and random sampling (870 females examined).

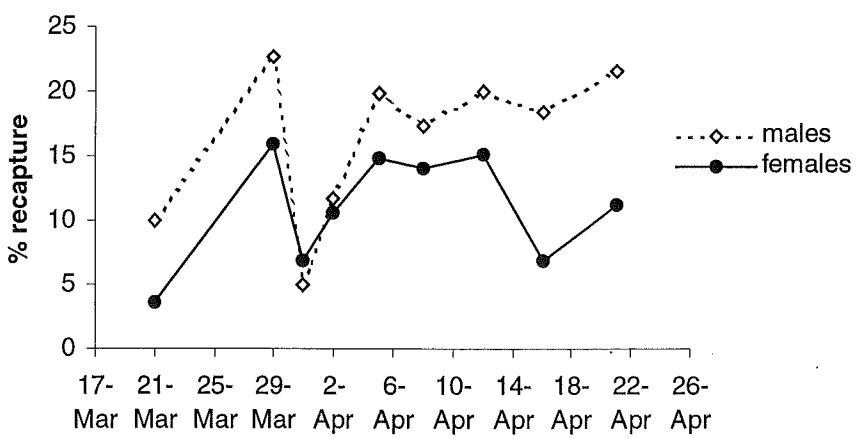


Figure 2.8 Recapture rate of males and females of *Hemigrapsus sexdentatus* collected under marked stones in March and April 1999 (644 males, 935 females examined).

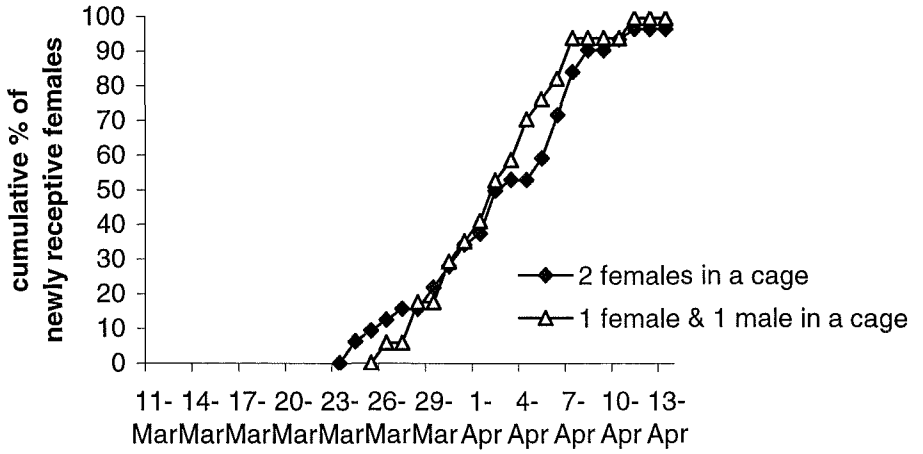


Figure 2.9 The cumulative percentage of caged female *Hemigrapsus sexdentatus* becoming receptive during the breeding season 2000. Data obtained from females placed in cages before the breeding season.

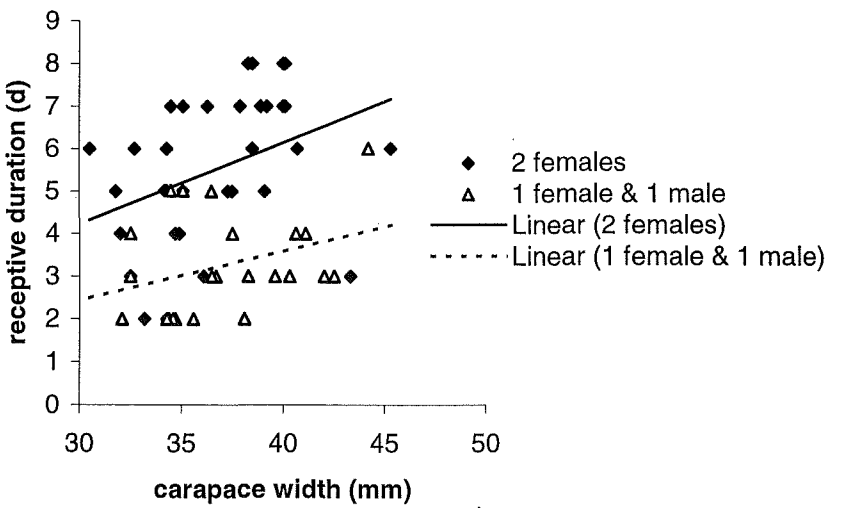


Figure 2.10 Duration of female receptivity of *Hemigrapsus sexdentatus* in relation to size and in the presence and absence of males in field cages in 2000. Linear regression equation: y = 0.1926x – 1.5652, R2 = 0.1371 (two females); y = 0.1152x – 1.0178, R2 = 0.113 (one female and one male).

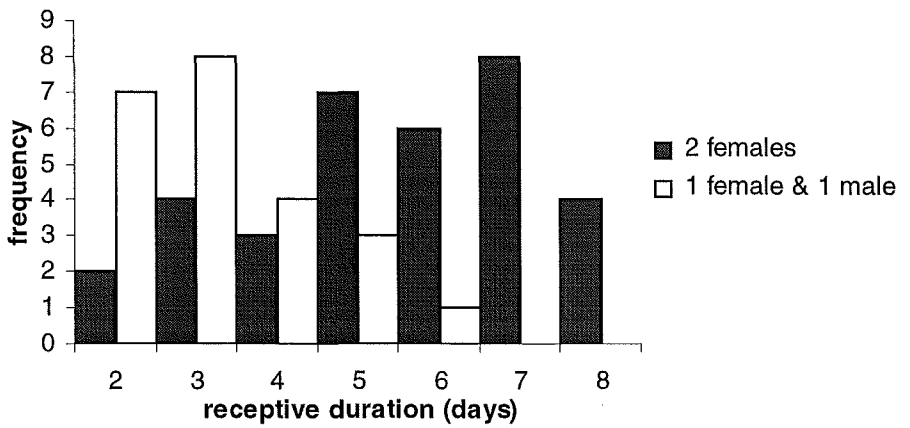


Figure 2.11 Frequency of duration of female receptivity of *Hemigrapsus sexdentatus* in the presence (n = 34 females) and absence of males (n = 23 females) in field cages in 2000.

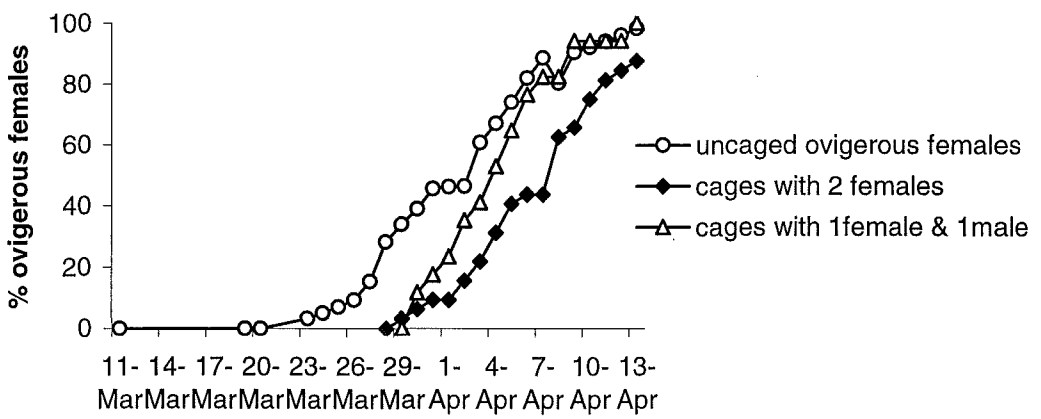
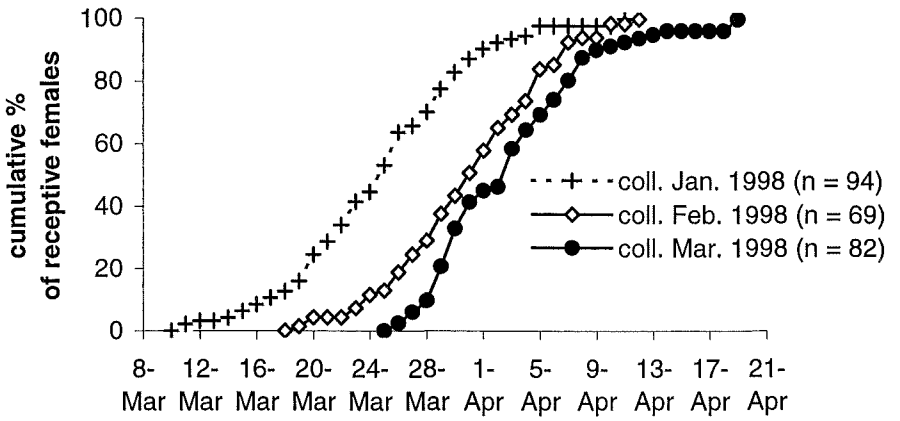


Figure 2.12 Percentage of ovigerous females of *Hemigrapsus sexdentatus* held in cages in the presence and absence of males and uncaged females from a natural population during the breeding season in 2000. Data for caged females were obtained from cages placed in the field before the breeding season.

A.



B.

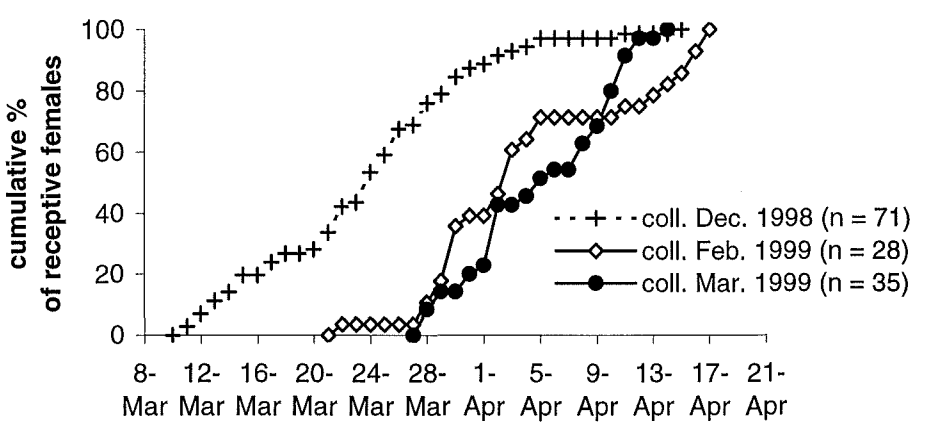


Figure 2.13 The cumulative percentage of females becoming receptive in the laboratory during the breeding season of *Hemigrapsus sexdentatus* in 1998 and 1999. A. Females collected in 1998; B. females collected in 1998 and 1999.

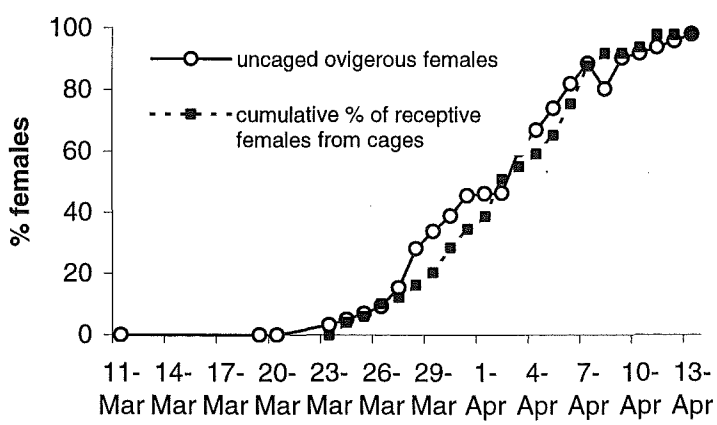


Figure 2.14 Comparison of percentage of ovigerous females of *Hemigrapsus sexdentatus* of the free (uncaged) natural population and the cumulative percentage of receptive females placed in cages before the start of the breeding season in 2000.

3 Mating behaviour, female receptivity and male-male competition in the intertidal crab *Hemigrapsus sexdentatus* (Brachyura: Grapsidae)

Abstract - Female receptivity and male-male competition was studied in a laboratory population of the New Zealand rock crab *Hemigrapsus sexdentatus*. Mating only occurred in the few days prior to oviposition, when females have mobile gonopore opercula. No courtship behaviour was observed. After mating successfully, males guarded females constantly and mated with them repeatedly until the females laid eggs, unless the male was displaced by another male. Isolated females remained receptive for significantly longer than females housed temporarily or constantly with males in the laboratory. This suggests that females have some control over the duration of their receptive period. Female attractiveness changed during the receptive period such that approaches by males and mating frequency decreased over time. The duration of female receptivity and mating frequency was independent of female size. Male-male competition was high with frequent attacks on pairs by other males, which resulted in either the female escaping (56.2%), the pair remaining together (36.5%), or take-over by the attacking male (7.3%). Larger males mated significantly more often than medium and small males and were more likely to take-over a female from another male. Large males were also more successful than small males even when size difference between the two competitors was small. Post-copulatory guarding appears to deny competitors access to the female and reduce the risk of sperm competition. Although male-male competition appears to be the dominant factor in pair-formation in this species, the ability of females to extend their receptivity in the absence of males, will have an impact on the extent of male-male and sperm competition.

3.1 Introduction

Reproductive strategies in Crustacea are the result of a complex process in which mating behaviour plays an important role. Although numerous aspects of mate choice, male-male competition and sperm competition (defined as competition between ejaculates of two or more males for the fertilisation of the ova of a single female; Parker 1970) have been extensively studied for birds and insects (see reviews of Smith 1984; Anderson 1994; Birkhead & Møller

1998; Eberhard 1996), these processes are relatively poorly understood for most Crustacea. For example, it is often not known to what extent female choice (i.e., before and after copulation) exists and how it influences male-male and sperm competition in Crustacea.

Much of the information available on decapod mating behaviour, sperm storage and sperm competition comes from studies on either commercially important species, such as edible crabs and lobsters, or ocypodid crabs. Studies on commercially important species were often undertaken to understand the reproductive biology of a species to avoid over-exploitation and to estimate the quantitative and qualitative impact of size-selective or gender-selective fisheries on reproductive success. For example, aspects of the reproductive biology have been studied in the blue crab *Callinectes sapidus* (Portunidae) (e.g., Gleeson 1980; Smith 1992; Jivoff 1997a,b; Jivoff & Hines 1998a,b; Kendall & Wolcott 1999), the spider crabs *Chionoecetes opilio* (e.g., Beninger et al. 1988, 1991, 1993; Sainte-Marie & Lovrich 1994; Moriyasu & Comeau 1996; Sévigny & Sainte-Marie 1996; Moriyasu & Benhalima 1998; Sainte-Marie & Sainte-Marie 1998; Urbani et al. 1998; Sainte-Marie et al. 2000) and *C. bairdi* (e.g., Adams & Paul 1983; Paul 1984; Paul & Adams 1984; Donaldson & Adams 1989; Paul & Paul 1992, 1996; Stevens et al. 1993), the stone crab *Hapalogaster dentata* (Goshima et al. 2000), spiny lobsters *Jasus edwardsii* and *Panulirus argus* (MacDiarmid & Butler 1999) and fiddler crabs *Uca* spp. (e.g., Christy & Salmon 1984; Murai et al. 1987; Medina 1992; de Rivera and Vehrencamp 2001), to name a few.

An overview of competitive mating, mate choice and mating associations of brachyuran crabs was given by Christy (1987), on sperm competition and the evolution of mating behaviour of Brachyura by Diesel (1991), and on spermatophores and sperm transfer in marine crustaceans by Subramoniam (1993). In comparison, only limited information is available on factors influencing the reproductive success of intertidal grapsid crabs.

Mating in Brachyura can be either restricted to a certain period of time when females are morphologically able to mate (e.g., postmoult mating in several Portunidae and Cancridae) or when their gonopore opercula become temporarily decalcified during the intermoult (e.g., some Grapsidae and Ocypodidae), or it can be unrestricted in time and females are morphologically able to mate at any time (e.g., some Grapsidae, Majidae and Ocypodidae; Hartnoll 1969; Diesel 1991). However, for the latter, seasonal mating peaks can occur and are often linked with the oviposition period (Diesel 1986; Skinner & Hill 1987; Jones & Hartnoll 1997).

Some species with gonopore opercula appear to have permanently mobile opercula which allows mating at any time (e.g., *Cyclograpsus integer* (Hartnoll 1965), *Uca vocans* (Salmon 1984), *U. lactea* (Murai et al. 1987)), whereas others exhibit seasonal changes in opercula

mobility and are therefore restricted in their mating activities (e.g., *U. pugilator* (Christy 1982), *U. pugnax* (Greenspan 1982), *Sesarma* sp. (Zimmerman & Felder 1991)). Decalcification of the gonopore opercula, the process of an immobile opercula becoming mobile, was found to occur before and after pairing in the days prior to spawning for the ocypodid crabs *U. pugnax* (Greenspan 1982) and *Ilyoplax pusilla* (Henmi & Murai 1999). Most ovigerous females had recalcified opercula before larval release (Henmi & Murai 1999). In the grapsid crab, *Gaetice depressus*, decalcification occurred after moulting and shortly before oviposition, and the opercula remained mobile for one to three days (Fukui 1993). The timing and duration of gonopore decalcification is important, as this is the time females are receptive and engage in mating activities. The duration of female receptivity in turn determines the operational sex ratio (receptive females per mature males), which has an impact on the extent of male-male competition.

Studies on male-male competition in crabs have demonstrated that male size is often a major factor in male success (Berril & Arsenault 1982; Abele et al. 1986; Donaldson & Adams 1989; Jormalainen et al. 1994; Reid 1994; Moriyasu & Comeau 1996; Koga & Murai 1997; Jivoff 1997b; Jivoff & Hines 1998a; Sainte-Marie et al. 1999). Male-male competition for receptive females occurs typically before or after moulting and during the time of oviposition and is often evident as pre-copulatory or post-copulatory guarding. Males guard to protect soft-shelled females from predation and cannibalism (when mating occurs shortly after the female moults), to prevent other males from mating with the female (Jivoff 1997a), and to prevent sperm competition within the female's spermatheca (see below). The time males spend guarding the female can be considerable and costly. For example, males of the portunid crab *Arenaeus cribrarius* carry the females under themselves for 2 months, which includes 30 days before and 30 days after copulation (Pinheiro & Fransozo 1999). However, in many other species post-copulatory guarding is limited to a few days, as in *C. sapidus*, where males guard females usually for 48 to 96 hours after moulting and mating (Jivoff 1997a). In addition, male *Gammarus lawrencianus* showed a reduced growth of 45% when paired (amplexus) compared to unpaired males (Robinson & Doyle 1985). Furthermore, it has been shown that pairing can increase the risk of predation in some taxa (Magnhagen 1991), but this is not necessarily the case for other species (Gwynne 1989). For example, it has been shown that an elevated predation risk changes the mating behaviour and courtship of the fiddler crab *Uca beebei* (Koga et al 1998) and duration of precopula in the amphipod *Hyalella azteca* (Strong 1973).

The occurrence of sperm competition seems likely for many crabs as females often store viable sperm for several broods, over long periods, and through moulting (Greenspan 1982; Paul

1984; Salmon 1987; Fukui 1990; Yamaguchi 1998), and are capable of multiple matings within a single reproductive period (Diesel 1989; Donaldson & Adams 1989; Orensanz et al. 1995; Jivoff 1997a; Gonzáles-Gurriarán et al. 1998; Urbani et al. 1998).

The intertidal crab *Hemigrapsus sexdentatus* (formerly known as *H. edwardsii*) is endemic to New Zealand, where it occurs on relatively sheltered rocky or stony shores (McLay 1988). *Hemigrapsus sexdentatus* has one short and highly synchronised breeding season every year, in which all mature females lay eggs within about three weeks (end of March to beginning of April). Females of *H. sexdentatus* have gonopores that are covered by opercula. For most of the year, these opercula are calcified and immobile, which inhibits any mating activities. The sex ratio of the mature population is usually slightly female biased, however the operational sex ratio is highly male biased (Chapter 2).

The aim of this study was to describe the general mating behaviour of *H. sexdentatus* in the laboratory and to examine to what extent male-male and sperm competition occur. In particular, I wanted to test whether male size influences mating success and whether males display behaviour that ensures their paternity and reduces the risk of sperm competition. Furthermore, I wanted to investigate whether females have any control over the duration of their receptivity. For example, females could try to extend the duration of their receptivity if males are absent or shorten it when they had a sufficient number of matings.

3.2 Materials and Methods

Hemigrapsus sexdentatus were collected randomly by hand at an intertidal boulder field in Canterbury, South Island, New Zealand (43° 06′ S, 172° 53′ E) on 15 January, 15 February, 15 March, 13 April, and 18 December 1998, 18 February, 10 and 21 March 1999, 15 March, and 7 April 2000. All crabs were measured (carapace width (CW), using a Mitutoyo digital callipers to the nearest 0.1 mm), sexed (using the relative abdomen width; females have a wider/broader abdomen than males), and the reproductive stage of the female determined (i.e., ovigerous or not, and whether the gonopore opercula were mobile, see below). Mature crabs (carapace width (CW) larger than 24 mm) were taken to the laboratory, where they were held under a 12 h light: 12 h dark cycle in tanks with circulating seawater of 12 - 15°C. Males and females were kept in separate group tanks and fed opened blue mussels (*Mytilus edulis*) three times a week unless otherwise stated. In addition, field observations were carried out during the breeding season in March and April of 1999 and 2000 (see below).

To assess female receptivity, which is a prerequisite for assessing the time of mating, the gonopore opercula of all captive females were probed weekly from January 1998 and December 1998 until the end of February and daily in March and April in 1998 and 1999 (which coincides with the time before and during the breeding season). To determine operculum mobility, the abdomen was slightly lifted and one of the two opercula was probed carefully with fine forceps. When the opercula were mobile, and could be pushed inwards like a trapdoor, females were considered receptive. If the opercula were immobile and could not be moved, females were counted as being non-receptive. Ovigerous females had mobile opercula up to four days after oviposition, but were assumed to be non-receptive, as matings were never observed in the laboratory once a female had laid eggs (see below and results). Females were individually marked on their first receptive day with small, numbered bee tags (round plastic discs of 3 mm diameter, glued to the carapace with cyanoacrynalat glue).

Female receptivity in the absence and presence (temporary or constant) of males

Daily male exposure (short-term trials)

To examine the receptivity and mating behaviour of females during short encounters with males, a series of short-term experiments were carried out each day during the receptive period of females. One female and two different sized males were placed in a tank and their behaviour was observed. Large males had a CW between 35.1 and 51.7 mm and small males between 29.4 and 45.0 mm. The mean size difference between males was 9.9 mm. The large males selected for those experiments were always larger than the female. The tank (25 cm long × 25cm wide × 25 cm high) was filled with seawater (12 - 15°C) to a depth of 15 cm, contained one rock for shelter and was kept at ambient room temperature (about 19°C). The crabs were observed for a minimum of one hour each day. If a female mated within this first hour, the male she mated with was removed after copulation and the remaining two crabs were observed for another hour to see whether the second male mated with the female. After a maximum of two hours crabs were separated and returned to their group tanks. Males that mated were usually not used in the following two days to avoid sperm depletion and a decrease in male activity. However, it is likely that this species has adapted to a short synchronous mating season by having a large store of spermatophores and sperm fluid (see also results). Notes were taken on the general mating behaviour, and the frequency and duration of copulation. A total of 71 females were observed on each day during their receptive period, resulting in 297 trials.

Constant male exposure (long-term trials)

To examine mating behaviour over the entire receptive period of individual females, long-term experiments were carried out starting on the female's first receptive day and continuing until she laid eggs using continuous video recording (recorder on 24 hour time-lapse mode, 0.18 sec video recording interval). For each trial, one receptive female and three different sized males (large males, CW ≥ 40.0 mm; medium males, CW 34.0 – 39.9 mm; small males, CW < 34.0 mm) were placed in a glass tank. The tank (51 cm long × 25cm wide × 25 cm wide) was filled with seawater to about 15 cm depth, contained two rocks for shelter, and was kept in a 15°C constant temperature room with a 12 hour light-dark cycle. Infrared light was used during night hours to allow video recording. The water was changed daily using plastic tubes for carefully draining and refilling to keep disturbance for the crabs to a minimum. One to two hours before the water change two opened blue mussels were placed in the tank, and then removed just before the tanks were refilled. Fourteen females were monitored continuously resulting in a total of 1909 hours of observation. When the recordings were viewed, the time, frequency and duration of mating, guarding and attacks by males on pairs were recorded.

No male exposure

To examine the duration of female receptivity in the absence of males, 74 females were kept isolated and their receptivity monitored. Females were held in groups of 20 - 30 under a 12 h light : 12 h dark cycle in tanks with circulating seawater of 12 - 15°C.

Other mating behaviour observations

To investigate whether ovigerous females mate, 60 ovigerous females were placed in a tank with two males (large and small males as in short-term experiments above) on the day of egg laying (n = 20), and one (n = 20) and two (n = 20) days after oviposition and observed for one hour.

To examine whether males can distinguish between non-receptive and receptive females, a male was placed in a tank with a receptive and a non-receptive female (N = 22) and their behaviour observed for one hour. In addition, a male was placed in a tank with two non-receptive females (N = 22). The glass tanks and general set up for both experiments was the same as for the short-term experiments (see above).

To test whether the difference in male size influences male success, one receptive female and two males were placed in a tank and observed for one hour. Receptive females were randomly chosen on any of their first four receptive days. The set up for this experiment, including the size range of large and small males, was identical as for the short-term experiments

above and data concerning the male-male competition in relation to differences in male size were combined. A total of 169 trials where matings were observed were analysed and included male size differences of 3 - 4 mm (n = 26), 5 - 8 mm (n = 43), 9 - 12 mm (n = 55), 13 – 16 mm (n = 34), and 17 – 22 mm (n = 11).

To examine the pairing pattern with regard to male size in the field, the field site was extensively searched for paired crabs (males guarding a female) during the breeding season in March and April in 1999 and 2000 (see Chapter 2). The carapace widths of the paired crabs and the reproductive state of the female were noted.

Sperm storage and ejaculate size

To investigate whether females mate during each breeding season, the spermathecae of ovigerous females collected from the field in April 1998 and 2000 were examined and weighed. To estimate whether mating activities are similar in the field and laboratory, the weight of these spermatheca was compared with those of ovigerous females held and observed in the laboratory (i.e., with a known number of matings) and with females held in field cages in a one female to one male ratio, during the 2000 breeding season (see Chapter 2).

To examine whether changes in the weight of the vasa deferentia of males (where spermatophores and sperm fluid are stored) occur during the mating season, males were collected from the field one week before and 2 weeks after the breeding season in 2000 and their vasa deferentia removed and measured.

To estimate the ejaculate size transferred to empty spermathecae (first ejaculate), the weight difference between spermathecae which were filled during one copulation and the calculated weight of the empty spermathecae of a given female size was calculated. Similarly, to estimate the size of the second ejaculate, the weight difference between spermathecae which were filled during two copulations and the calculated weight of the spermathecae after one mating of a given female size was calculated. The relationship between the empty spermathecae weight after one mating and female carapace width was determined using linear regression. In six cases negative values were calculated for the second ejaculate, which were not used in the statistical analysis comparing first and second ejaculate. In excluding these six negative values, the average weight of the second ejaculate was increased slightly. However, the first ejaculate was still significantly larger than the second (see results).

Crabs were killed by placing them in a freezer at -15°C for about 1 hour. The carapace and the upper internal organs were removed to expose the gonads. Both spermathecae of each female were examined and dissected out by a cut close to the gonopore. Spermatheca were

considered full when they were clearly visible as two large, round, fully filled 'balloons' as soon as the overlying internal organs were removed. The vasa deferentia of the males were removed and weighed. The weight of the spermathecae (two combined from each female) and the two vasa deferentia was determined to the nearest 0.1 mg.

Terminology and Statistics

Females of *H. sexdentatus* are referred to as being receptive, when they have mobile gonopore opercula prior to oviposition. The duration of female receptivity is defined as the time from the first day a female has mobile gonopore opercula up to the day she lays eggs. 'Empty' spermathecae are defined as spermatheca of unmated females, meaning they have not mated in the current mating season. However, these 'empty' spermatheca do contain some sperm stored from the previous mating season. Mean values given are followed by the standard error of the mean unless otherwise stated. Data were analysed using SYSTAT 9.

3.3 Results

General mating behaviour

The gonopore opercula of females were mobile during the intermoult stage a few days before and after oviposition. Only females with mobile gonopore opercula were observed to mate. Matings were observed from the first receptive day onwards until oviposition. No matings were observed after oviposition although the gonopore opercula remained mobile for another two to four days. Females and males showed no obvious courtship behaviour. Typically, a male quickly approached a receptive female, grasped her with his chelipeds, and manoeuvred the female with his chelipeds and legs to face her. The male then opened its abdomen while moving under the female and pulling her over him. This caused the pair to fall backwards onto the male's back and resulted in the typical female-on-male position (Fig. 3.1a). By then the male had positioned his abdomen underneath the female's abdomen, and the gonopods were placed onto the female gonopores. The process, from initial contact to copulation, took usually only a few minutes, but could occasionally take several hours if the female resisted the mating attempts by the male. Females escaped frequently and quickly ran away. If the male mated successfully with the female, he would then guard her constantly and mate with her repeatedly until she laid eggs unless he was displaced by another male. Males guarded a female by holding one of her walking legs with his chelipeds and/or covering her between his legs (Fig. 3.1b). Females

appeared inactive during pair formation and postcopulatory guarding, unless they tried to resist the initial mating attempts by the males or tried to escape after copulation. Females were also inactive when another male tried to disrupt an established pair. The defending male would try to hold on and keep control over the female while fighting the other male. Typically, the female did not move much herself, but rather got dragged around and pulled back and forth while the males were fighting. Occasionally, the female lost a limb or received other damage to her exoskeleton during such fights. Females tried to escape during the fight presumably to avoid injury.

Males housed with two non-receptive females did not approach these females (n = 22). When given a choice, males initially had difficulty distinguishing between receptive and non-receptive females. Males approached non-receptive females first in 10 out of 22 experiments (45.5%) and held them shortly with their chelipeds before releasing them again. However, they only tried to mate (5 out of 22), and succeeded in mating (12 out of 22) with receptive females. Males therefore only approached non-receptive females in the presence of receptive females, which presumably release some chemical cues. If no chemical cues are present, they did not approach non-receptive females. Males guarded the females after mating until the end of the one-hour observation period. Post-copulatory guarding occurred whether or not another male was present.

In the laboratory, females mated with males that were larger or smaller than themselves. In the field receptive females were guarded by males, which were all larger than themselves (paired t-test: $t = 6.266$, $df = 10$, $P < 0.001$) and from the upper half of the size range of mature males (≥ 39.5 mm CW) (Fig. 3.2). However, there was no correlation between male and female size (linear regression: $R^2 = 0.171$, $F_{1,9} = 1.852$, $P = 0.207$). Five of the eleven paired females were found as single pairs underneath a rock. The other pairs were found under a rock together with either another male, an ovigerous female with mobile opercula, or two non-receptive females. Mating was never observed in the field despite intense searching of the field site and examination of 1490 females during three mating seasons. This was most likely due to the fact that mating itself lasts only about 10 minutes. Isolated females eventually laid fertile eggs without mating, presumably using stored sperm from the previous mating season (see also sperm storage below).

Female receptivity in the absence and presence of males and mating frequency

Females were receptive on average for 6.3 days when isolated, for 5.2 days when males were temporarily present every day, and for 5.5 days when males were constantly present over the

entire receptive period (Table 3.1). A significant positive correlation was found between the receptivity duration and female size when males were temporarily present, but not when males were absent or constantly present (Table 3.1). The duration of female receptivity was significantly longer when males were absent compared to present (ANCOVA: males temporarily and constantly present combined, female size as covariant, $R^2 = 0.126$, $F_{1, 156} = 16.57$, $P < 0.001$). This suggests that isolated females can prolong receptivity to increase the probability of mating prior to oviposition, a finding previously found using caged females on the study plot in the field (Chapter 2).

About half the females mated for the first time on the first day of their receptive period (47.9%). Others mated for the first time on the second (33.8%), third (11.3%), or fourth (7.0%) day when males were temporarily present. Mating frequency decreased significantly over the receptive period (Fig. 3.3). A positive correlation was found between the duration of receptivity and the number of matings when males were temporarily present every day (Table 3.1). The longer the receptive period the more often females mated. In contrast, no correlation was found between the duration of receptivity and the number of matings when males were constantly present (Table 3.1). A longer receptive duration did not increase number of matings significantly as females mated already relatively often in the first few days of the receptive period.

The mean number of matings of females was significantly higher when males were constantly present (7.8 matings) compared to when males were only temporarily present for a short time every day (2.7 matings) (ANOVA: $R^2 = 0.526$, $F_{1,83} = 92.24$, $P < 0.001$), most likely because the former have more time available for mating. Mating duration was positively correlated with female size, but did not differ significantly when males were temporarily (9.8 min) or constantly present (9.4 min)(Table 3.1). Mating duration did not change with subsequent matings (i.e. first, second and third mating of a female). Mating frequency was independent of female size (Table 3.1).

The time of day (day versus night) had no significant effect on the mating frequency (paired t-test: $t = -1.75$, $df = 13$, $P = 0.104$) in the trials where males were constantly present. Out of a total of 109 matings, 43 matings occurred during the day and 66 matings during the night.

Male-male competition

Males temporarily present

A total of 189 matings were observed in 297 short-term trials that were carried out on each day individually marked females were receptive. Females mated either not at all (n = 160), once (n = 85) or twice (n = 52) per trial. Large males were in 54.1% (46 out of 85) the only one to mate if the female mated only once ($x^2 = 1.15$, $df = 1$, $P = 0.283$), and significantly more often the first male to mate if the female mated twice (37 out of 52, 71.2%) ($x^2 = 18.62$, $df = 1$, $P < 0.001$). The total number of matings was however similar for large (98 matings) and small males (91 matings), most likely because the first male to mate was removed after copulation, giving the second (i.e., smaller) male an easier opportunity. Smaller males mated in 44.6% of the cases when the larger male was removed (37 out of 83), and 27.8% of the larger males mated after the smaller male was removed (15 out of 54). In both cases females were likely to mate again when the first male left, indicating high competition for the female.

The same trend was found when I investigated the mating frequency of males competing with other males of varying size differences in short-term experiments (N = 169). Large males were again more often the only (61 out of 103, 59.2%) or first male to mate (47 out of 66, 71.2%), and the total number of matings was similar for large (127 matings) and small males (108 matings). In addition, it was shown that large males obtained generally more matings almost irrespective of the size difference between the large and small male, i.e. whether it was only 4 or 20 mm carapace width difference (Fig. 3.4), suggesting that there is a strong selection for male size.

Males constantly present

There was no difference in the duration of matings by males of different sizes (ANCOVA, female size covariate, $R^2 = 0.001$, $F_{2,106} = 0.059$, $P = 0.943$). However, larger males mated significantly more often than medium and small males (ANOVA: relative male size, $F_{2,39} = 7.413$, $P = 0.002$; Tukey test: L *vs.* M, $P = 0.049$ and L *vs.* S, $P = 0.001$) (Fig. 3.5). No significant differences in mating success were found between medium and small males (Tukey test: $P = 0.377$). Large males were more likely to be the last to mate before the females laid eggs compared to medium and small males (64% compared to 14% and 21%, respectively). Male size had a significant effect on being the last male to mate (Chi-square test: $\chi^2 = 6.14$, $df = 2$, $P = 0.046$). Large males were the last to mate in 9 out of 14 cases compared to 2 times for medium males and 3 for the small males.

Frequently, one or two males simultaneously attacked a pair that was either mating (Fig. 3.1c) or where the male was guarding the female. A total of 219 attacks on pairs were observed that resulted in either the separation of the pair and the female escaping (56.2%), the pair withstanding the attack and remaining together (36.5%), or one attacking male taking over the female (7.3%). Defenders were mostly large males (51.6%), followed by the medium (33.8%) and small males (14.6%), whereas the probability of being an attacker was comparable for all male sizes (small males 28.3%, medium males 40.9%, large males 30.7%). Out of the 16 take-overs, most were won by large males (n = 12) followed by medium (n = 3) and small males (n = 1).

Sperm storage and ejaculate size

Isolated females were able to fertilise their ova using at least one-year-old sperm stored in the spermatheca from the previous mating season. In addition, females must have been able to retain sperm throughout the moulting cycle, as mature females moult before the next breeding season, typically shortly after the larvae hatch (personal observation).

The spermatheca weight increased with female size whether females were isolated (no matings) (linear regression: $R^2 = 0.707$, $F_{1,60} = 144.52$, $P < 0.001$), mated once (linear regression: $R^2 = 0.837$, $F_{1,28} = 143.44$, $P < 0.001$) or twice (linear regression: $R^2 = 0.751$, $F_{1,16} = 48.38$, $P < 0.001$) in the laboratory or had been collected as ovigerous females (mated unknown times) in the field (linear regression: $R^2 = 0.801$, $F_{1,52} = 209.69$, $P < 0.001$) (Fig. 3.6). The number of matings had a significant effect on spermathecae weight (ANCOVA: $R^2 = 0.804$, $F_{7,180} = 27.235$, $P < 0.001$). The spermathecae of unmated females where significant lighter than females that had mated one to six times or mated in the field (unknown number of matings) (Fisher's LSD: $P < 0.001$ for all). After the first mating the spermatheca weight increased only slightly with second and further matings (Fig. 3.6), and the spermathecae weight of females that mated one, two or three times were not significantly different from each other (Fisher's LSD: not significant). Furthermore, females that mated two and three times had no significantly different spermathecae weight than females that mated four times. However, the spermatheca weight of females that mated once was significantly different from females that mated four, five or six times (Fisher's LSD: $P = 0.005$, $P = 0.016$, $P = 0.002$, respectively). It appears therefore, that the spermathecae are filled almost to their maximum during the first mating event in the breeding season and subsequent matings only add a small amount to the spermathecae (Fig. 3.6).

All ovigerous females in the field had full spermathecae indicating that they had all mated during the current mating season. Furthermore, field ovigerous females had significantly heavier

spermathecae than isolated (unmated) females from the laboratory (Fisher's LSD: $P < 0.001$) (Fig. 3.6). There was no difference between the spermatheca weight of field ovigerous females and females that mated once or twice in the laboratory (Fisher's LSD: $P = 0.736$ and $P = 0.114$, respectively). However, field ovigerous females had significant lighter spermatheca than females that mated three and four times in the laboratory (Fisher's LSD: $P = 0.039$ and $P = 0.001$, respectively). This suggests that females in the field often mate more than once, but it might be less common that they mate more than twice. Field ovigerous females (uncaged, see chapter 2) had significantly lighter spermatheca than females held with males in field cages during the breeding season (ANCOVA: $R^2 = 0.619$, $F_{1,45} = 34.88$, $P < 0.001$) (Fig. 3.7). Caged females most likely mated more often than uncaged females, possibly because they were receptive for longer (2 to 8 days) than uncaged females (less than a day) or because they could not escape repeated mating attempts by males (see Chapter 2).

The calculated size of the first ejaculate transferred to 'empty' (= unmated in current mating season) spermathecae increased with female size (linear regression: $R^2 = 0.564$, $F_{1,23} = 29.80$, $P < 0.001$) (range: 7.6 mg to 79.6 mg) (Fig. 3.8a) and was independent of male size (Fig. 3.8b) (linear regression: $R^2 = 0.004$, $F_{1,23} = 0.087$, $P = 0.77$). This means that males of different sizes were equally able to fill empty spermathecae and that there is no advantage in this regard for a female to mate with a larger male. The first ejaculate received by a female with empty spermathecae was significantly larger than the second ejaculate (ANCOVA: $R^2 = 0.341$, $F_{1,34} = 7.062$, $P = 0.012$).

The weight of the vasa deferentia increased with male size and was not significantly different before and after the mating season for uncaged field males (ANOVA: $R^2 = 0.733$, $F_{1,44} = 0.11$, $P = 0.741$) (Fig. 3.9). However, after the breeding season, the weight of the vasa deferentia of caged males held with a female were significantly different (lighter) from free field males collected before (ANCOVA: $R^2 = 0.673$, $F_{1,38} = 20.93$, $P < 0.001$) and after (ANCOVA: $R^2 = 0.714$, $F_{1,41} = 13.12$, $P = 0.001$) the breeding season (Fig. 3.9). This indicates that uncaged field males had a large reproductive storage that did not decrease significantly over the breeding season, and that caged males most likely mated more often than uncaged field males.

3.4 Discussion

General mating behaviour

Hemigrapsus sexdentatus mates during the intermoult prior to oviposition when the gonopore opercula are mobile. Other grapsid crabs such as *Hemigrapsus nudus*, *H. oregonensis*, *Sesarma* sp., and *Gaetice depressus* have also been observed mating shortly prior to oviposition (Knudsen 1964; Lindberg 1980; Zimmerman & Felder 1991; Fukui 1993). Knudsen noted that in a communal tank of *H. oregonensis* several males were only attracted to one particular female. These males repeatedly tried to mate with that one female and completely ignored all other females. Knudsen (1964) at the time, did not examine the female genital opening in detail and was therefore not aware that a morphological requirement such a mobile opercula might be responsible for female attractiveness to males (possibly along with other communication means such as pheromones). Although I have not examined females of *H. nudus* and *H. oregonensis* it is likely that within the same genus similar female structures such as operculate gonopores (and / or a pheromone) occur. I conclude from observations on *Hemigrapsus nudus* and *H. oregonensis* (Knudsen 1964; Lindberg 1980) and my own on *H. sexdentatus* and *H. crenulatus* (Chapter 4), that the overall mating behaviour of species within the genus *Hemigrapsus* is very similar in that these species: show no apparent courtship behaviour, males initiate copulation by approaching females, copulation takes about 10 minutes, males try to guard females after mating (remains to be shown for species other than *H. sexdentatus* and *H. crenulatus*), and females frequently escape and mate with several males in a short period of time.

I did not detect any reproductive behaviour that would advertise female receptivity and do not know how males were able to detect receptive females. It is possible however that female *Hemigrapsus* crabs release some chemical attractant (pheromone) to attract males when they are receptive as has been shown for other crustacean species (Christofferson 1978; Gleeson 1980).

Females were always guarded until oviposition in the long-term experiments. In addition, post-copulatory guarding occurred whether or not a competitor was present in the short-term experiments. Post-copulatory guarding in this species is not as necessary as in species where mating occurs with a soft-shelled, vulnerable female that would need to be protected. As most females mated multiple times in the short and long-term observations, it is therefore likely that post-copulatory guarding in this species is exhibited by the male mainly to ensure paternity and to deny other males access to the female and therefore reduce the risk of sperm competition. Against this putative benefit of reducing sperm competition, males have to bear the cost of increased predation risk while guarding the females due to an increase in conspicuousness and

vulnerability (e.g., Strong 1973; Magnhagen 1991), and must forgo opportunities to mate with other females.

In the laboratory, females mated with males of all sizes, relative to their own size (but when there was a choice, larger males were more successful), whereas in the field females were paired mainly with males larger than themselves. According to Ridley (1983), size assortative mating is likely to occur in species where larger females produce larger clutches, larger males are more successful in obtaining mates, and the duration of mating is relatively long. Although the first two of these criteria apply to many crabs, with the duration of mating being variable, size-assortative pairing has been found in some crabs (i.e., the ocypodid crabs *Uca tetragonon* (Goshima et al. 1996) and *Uca rapax* (Greenspan 1980), the stone crab *Hapalogaster dentata* (Goshima et al. 2000)), but not in others (i.e., the calappid crab *Matuta lunaris* (Perez & Bellwood 1989), *Cancer gracilis* (Orensanz et al. 1995) and the spider crab *I. phalangium* (Diesel 1988)). Consequently, it has been suggested for *I. phalangium* that the fitness advantage to males in choosing large females might be offset by the costs of longer search time and the loss of mating opportunities with smaller females (Diesel 1988). A similar situation is likely to apply for *H. sexdentatus*, where receptive females are spatially and temporally limited even during the peak of the mating season (see Chapter 2).

In contrast to the apparently passive behaviour of females of *Hemigrapsus* spp. during pair formation, females of the grapsid crab *Gaetice depressus* actively approach males in a selective way. Females of this species preferentially approach males that are larger than themselves. Furthermore, if mating did occur between a male smaller than the female, it was the male that had initiated pair formation (Fukui 1994). However, females of *G. depressus* also mate with a range of male sizes including larger and smaller males than themselves, in a pattern similar to *Hemigrapsus*.

Female receptivity in the absence and presence of males and mating frequency

Isolated females stayed receptive significantly longer than females that had access to males and had mated. This indicates that females can extend their receptive period to increase the probability of mating if necessary. The same pattern of a longer receptive period of isolated females compared to females held with males was also found for females observed in field cages (see Chapter 2). This is an important finding as females can therefore influence the operational sex ratio, which in turn will influence the intensity of male-male competition. The duration of receptivity of females was similar when males were either temporarily or constantly present, but females mated more often when males were constantly present. This is not surprising as females

had much more time available for mating. In addition, this result shows that females, given the opportunity, mated multiple times and did not reduce the receptive duration after one or two copulations.

Male-male competition

Overall, larger males were found to be more successful in the number of matings and female guarding. However, a substantial proportion of the matings occurred with males smaller than their competitors. Larger males have relatively larger and stronger chelipeds than smaller males and this most likely provides them with an advantage in handling the female and during male fights. In addition to being more successful in gaining access and mating with a female, as is the case with *H. sexdentatus*, larger males of *Callinectes sapidus* were found to pass larger ejaculates to females than smaller males (Jivoff 1997b), which is likely to increase their fertilisation success. However, this was not the case for males of *H. sexdentatus* (see below). In contrast to male size being a strong selective factor, the three morphs of the marine isopod *Paracerceis sculpta* have developed alternative mating strategies in which each morph follows its own reproductive strategy which gives them eventually equal mating success (Shuster & Wade 1991). In this species large males defend harems, medium size males mimic female behaviour and morphology, and small males establish themselves in large harems.

Sperm storage and ejaculate size

In the long-term experiments, large male *H. sexdentatus* were more often the last to mate before oviposition. This is likely to increase their chance to fertilise the ova, as female *H. sexdentatus* have a ventral-type spermathecae where the oviduct and vagina are at the ventral end of the spermatheca (see also Diesel 1991). In other species such spermathecae have been reported to favour the last male's sperm during fertilisation. For example, the last male to mate seals off the rival's sperm and fertilises most of the ova in *I. phalangium* (Diesel 1989, 1990). Similarly, over 90% of the eggs in the crab *Scopimera globosa* are fertilised by the last male (Koga et al. 1993). Males of the crab *Chionoecetes opilio* are also advantaged when they are the last to mate, due to the stratification pattern of ejaculates in the female spermatheca (Urbani et al. 1998). However, the storage pattern of the sperm could be disrupted in spermatheca with large loads, which can result in multiple-male paternity in *C. opilio* (Sainte-Marie et al. 2000).

It has been shown for the grapsid crab *Metopograpsus messor* that changes start to occur within the spermatheca shortly after copulation. Initially, spermatophores are clearly discernible within the spermathecae. After the first day, the spermatophore wall starts to disintegrate and

three days after copulation the spermathophore wall starts to completely dissolve causing spermatozoa to become dispersed in the spermathecal lumen (Anilkumar et al. 1999), which might eventually lead to the mixing of ejaculates. Similarly, spermatozoa within the spermathecae of *Chasmagnathus granulata* were found not to be stored in discrete packages and it was suggested that spermatozoa from different matings could mix (López Greco et al. 1999). However, the number of matings was unknown in the latter study and could therefore not be related directly. Investigations on the short and long term pattern of sperm storage are necessary for *H. sexdentatus* (which might cause sperm mixing or stratification), to establish the exact fertilisation process in this species.

All ovigerous females from the field had full spermathecae indicating that they all mated during the breeding season. It appears that most females in the field mate once or twice, some possibly 3 or 4 times, based on a comparison with spermathecae weight of females which mated in the laboratory. Females in the laboratory mated between one and 13 times in the short and long-term trials, and therefore appeared to mate more often than females in the field, possibly due to a longer receptive period compared to field females. The spermathecae weight increased with female size whether females had mated or not. An increase of spermatheca weight with female size was also observed for *Uca lactea* (Murai et al. 1987), *Callinectes sapidus* (Jivoff 1997b), *Macrophthalmus hirtipes* (Jennings et al. 2000), and *Hemigrapsus crenulatus* (see Chapter 4).

During the first mating in the season, male *H. sexdentatus* adjusted the ejaculate size according to the size of the female, i.e., transferred relatively large ejaculates to large females. Similarly, male *Panulirus argus* vary the amount of ejaculate positively with female size (MacDiarmid & Butler 1999). Furthermore, large and small male *H. sexdentatus* were equally able to transfer the first ejaculate to small and large females. However, this was not the case for *C. sapidus*, where smaller males transfer smaller ejaculates than larger males (Jivoff 1997a,b). It has been shown for *Chionoecetes opilio* and *Callinectes sapidus*, that larger ejaculates contain more sperm compared to smaller ejaculates (Sainte-Marie & Lovrich 1994; Jivoff 1997b). Although, I did not examine this relationship for *H. sexdentatus*, it is likely that a similar relationship between ejaculate size and sperm number would be found. In this case, larger males would therefore transfer more sperm to larger females which produce more eggs compared to smaller females.

In *H. sexdentatus*, the spermathecae were filled almost to their maximum after the first copulation and subsequent matings increased the spermathecae weight only slightly. This could be due to an increased resistance while filling the relatively full spermatheca any further. It

appears therefore that the ejaculate size decreases with subsequent matings due to the fullness of the spermathecae. Alternatively, males could try to flush out the previous contents to be able to get some of their ejaculate into the spermatheca, which has been reported for insects (see Birkhead & Møller 1998). However, this is not very likely, because the gonopods are too big to be inserted into the female spermathecae and a narrow vagina does probably not permit flushing of viscous ejaculates. It has been shown for *C. sapidus* that second males transfer larger ejaculates than first males, and third males more than the first but less than the second (Jivoff 1997a). In contrast to *H. sexdentatus*, *C. sapidus* spermathecae are therefore not filled almost to their maximum after the first mating but instead can hold several large ejaculates.

In summary, male *H. sexdentatus* search for and defend receptive females until they lay eggs. Although male-male competition appears to be the dominant factor in pair-formation in this species, the ability of females to extend their receptivity in the absence of males, will have an impact on the extent of male-male competition and sperm competition.

Table 3.1 Female receptivity and mating frequency of *Hemigrapsus sexdentatus* in the absence or presence of males. Mean values given are followed by the standard error of the mean. Results of linear regression are given in parentheses.

Parameters	Males absent (n = 74)	Males present temporarily (n = 71)	Male present constantly (n = 14)
Receptivity duration (d)	6.3 ± 0.2 (range: 3 to 11)	5.2 ± 0.2 (range: 2 to 10)	5.5 ± 0.4 (range: 3 to 9)
Receptivity duration vs. female size	no correlation ($R^2 = 0.004$, $F_{1,72} = 0.293$, $P = 0.590$)	positive correlation ($R^2 = 0.201$, $F_{1,69} = 17.33$, $P < 0.001$)	no correlation ($R^2 = 0.035$, $F_{1,12} = 0.44$, $P = 0.522$)
Receptivity duration vs. no. of matings	N/A	positive correlation ($R^2 = 0.185$, $F_{1,69} = 15.66$, $P < 0.001$)	no correlation ($R^2 = 0.17$, $F_{1,12} = 2.45$, $P = 0.143$)
Mating duration (min)	N/A	9.8 ± 0.4 (range: 3 to 22; n = 75)	9.4 ± 0.5 (range: 3 to 27; n = 109)
Mating duration vs. female size	N/A	-	positive correlation ($R^2 = 0.066$, $F_{1,107} = 7.547$, $P = 0.007$)
Mating frequency per female	N/A	2.7 ± 0.2^a (range: 1 to 6)	7.8 ± 0.8^b (range: 2 to 13)
Mating frequency vs. female size	N/A	no correlation ($R^2 = 0.029$, $F_{1,69} = 2.09$, $P = 0.153$)	no correlation ($R^2 = 0.005$, $F_{1,12} = 0.066$, $P = 0.802$)

N/A, not applicable; a,b, statistically significant differences

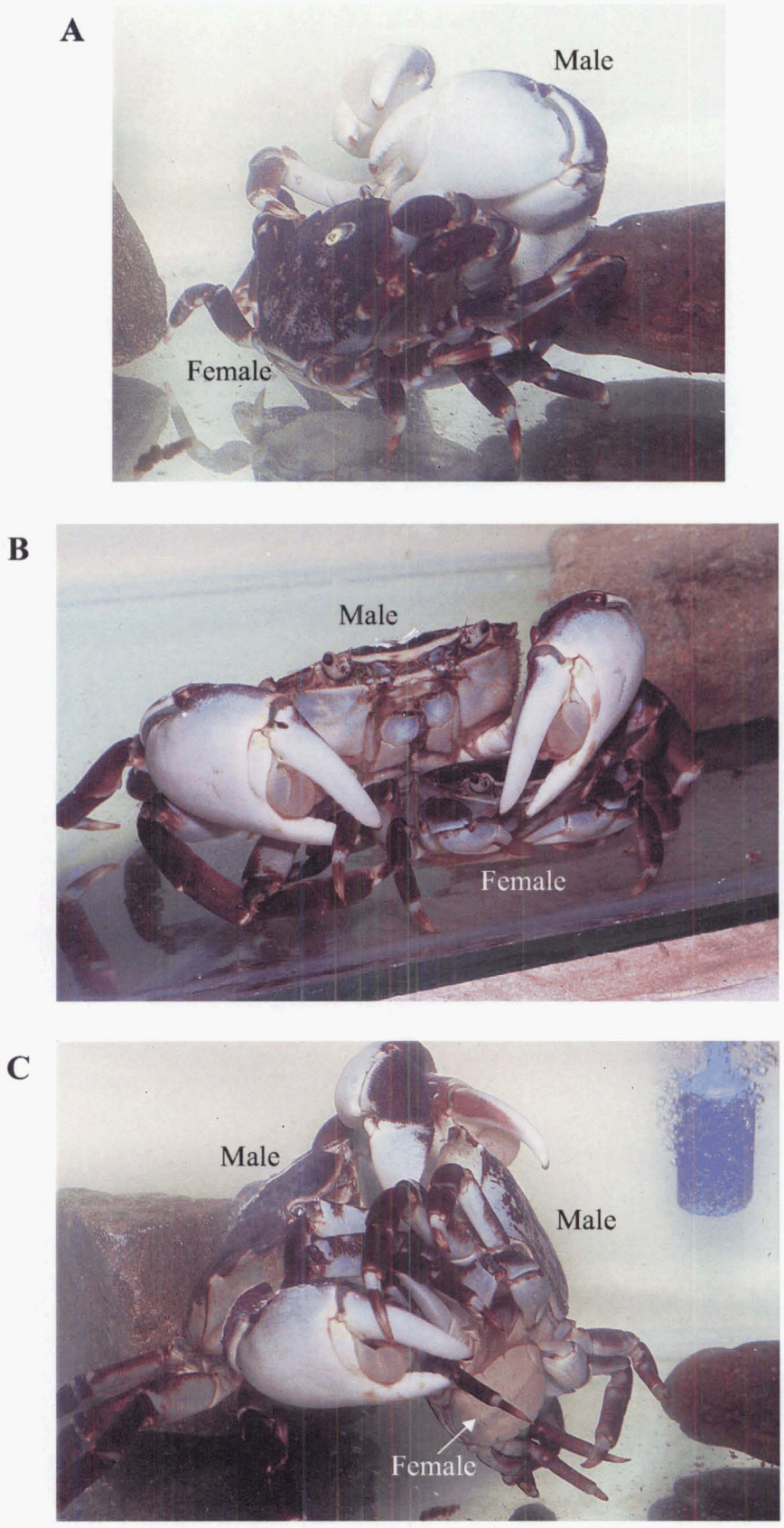


Figure 3.1 *Hemigrapsus sexdentatus*. A. Mating pair; B. Post-copulatory mate guarding (Note: Male holding on to female with his chelipeds and caging her between his walking legs); C. Large male (on left) attacking the mating pair (female in middle, male on right).

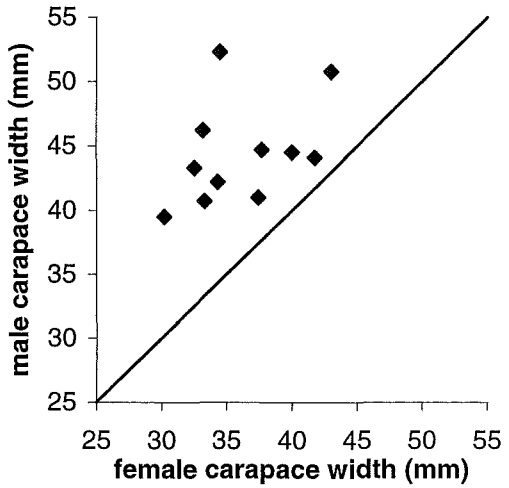


Figure 3.2 Size of male and female pairs of *Hemigrapsus sexdentatus* observed in the field. Line indicates equal male and female sizes.

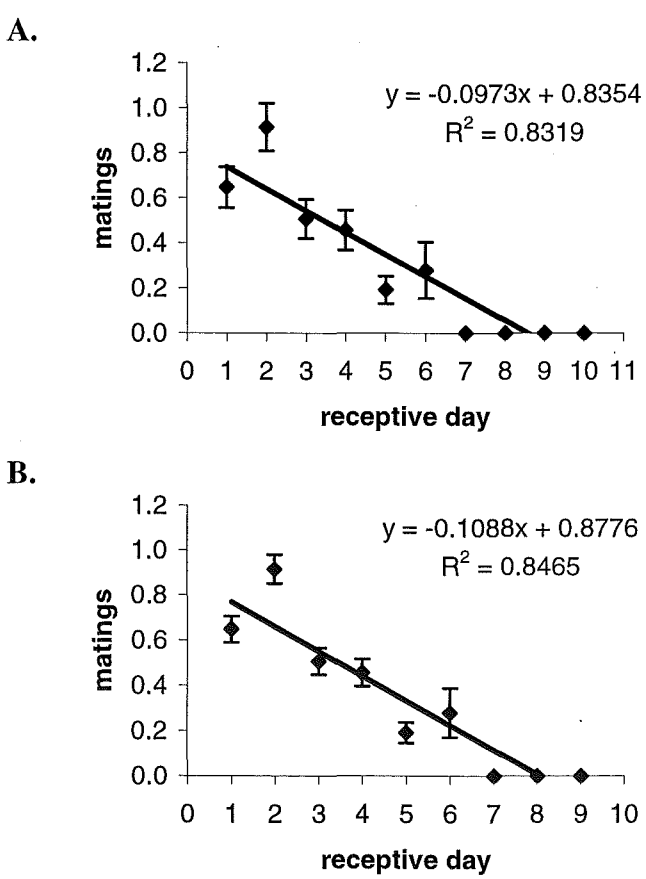


Figure 3.3 Mean number (± S.E.) of matings during each day of the receptive period of female *Hemigrapsus sexdentatus*. A. Males temporarily present (n = 71), linear regression: $P < 0.001$; B. Males constantly present (n = 14); linear regression, $P = 0.001$.

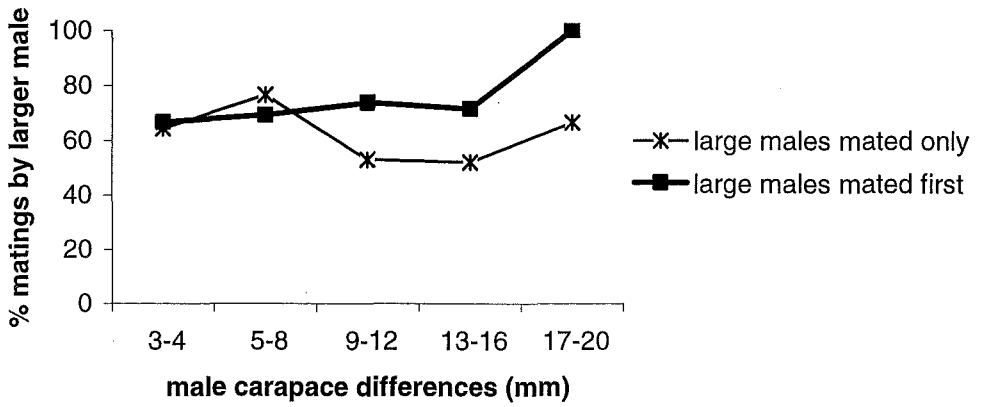


Figure 3.4 Percent matings of larger male *Hemigrapsus sexdentatus* competing with other males of varying size differences in short-term trials. The male that mated first was removed after copulation and the remaining male was left with the female for another hour. Data shown are from experiments where females mated once (n = 103) or twice (n = 66).

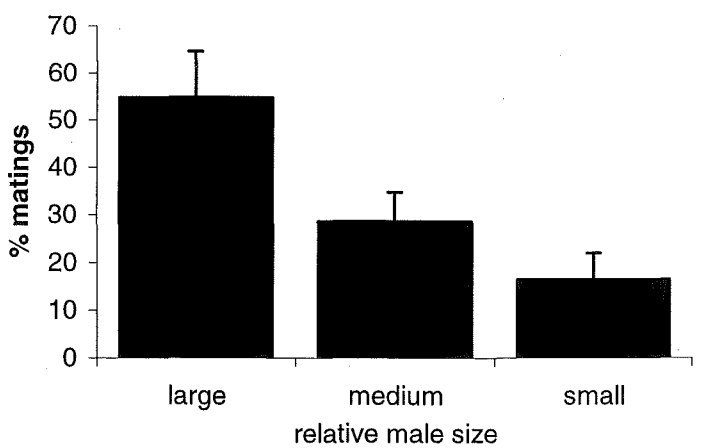


Figure 3.5 Relative mating success of large, medium and small male *Hemigrapsus sexdentatus* in the presence of one female in long term trials (n = 14).

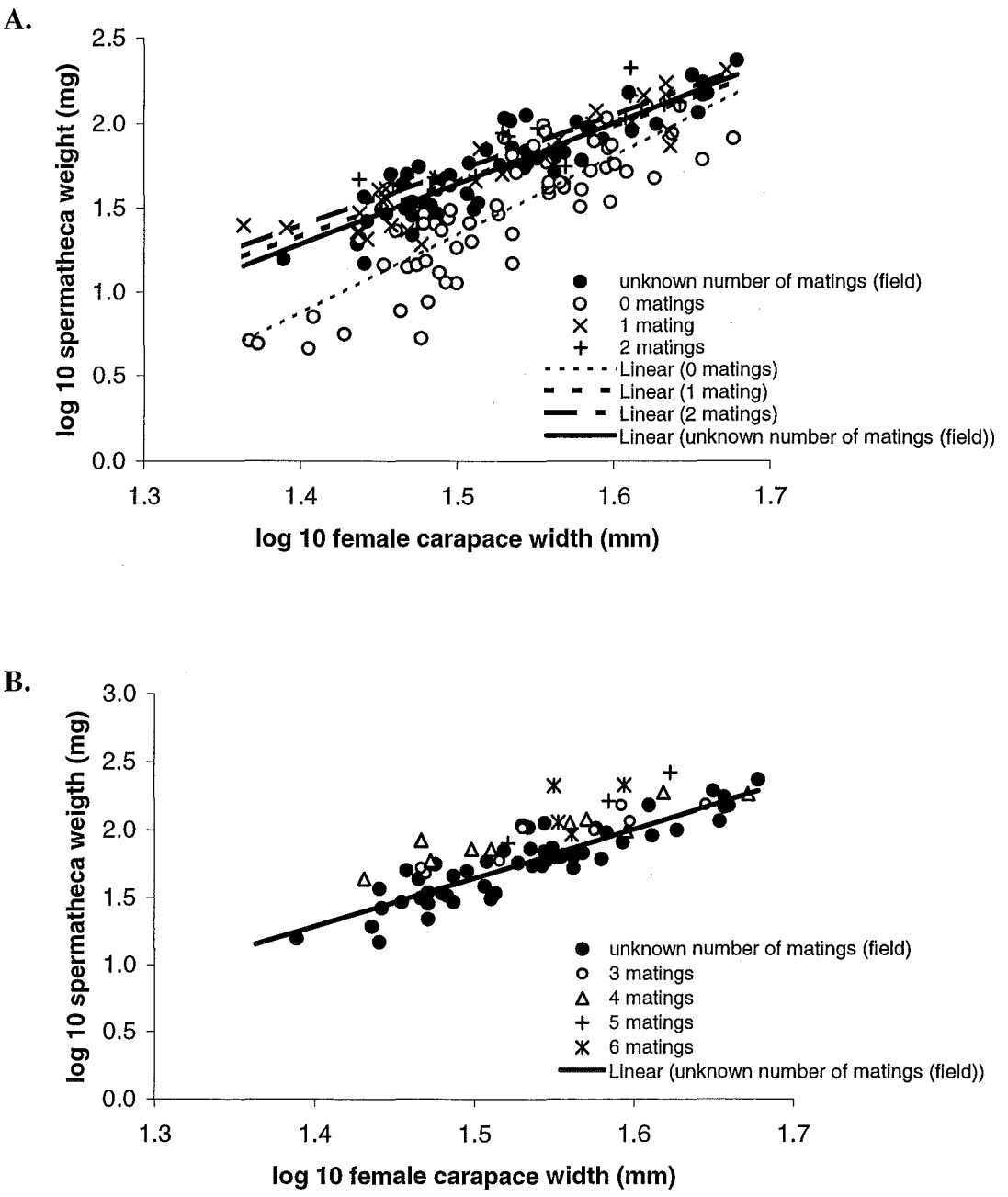


Figure 3.6 Spermatheca weight of ovigerous female *Hemigrapsus sexdentatus* from the field (1998) and laboratory. A. Field mated females compared to isolated females and females that mated once or twice in the laboratory; B. Field mated females compared to females mated three to six times in the laboratory. (M = number of mating; linear regression equations: no M (n = 62), y = 4.6948x – 5.6982; 1 M (n = 30), y = 3.3027x – 3.2938; 2 M (n = 18), y = 3.319x – 3.2537; 3 M (n = 8); 4 M (n = 11); 5 M (n = 3); 6 M (n = 3); field (unknown number of matings, n = 54), y = 3.5948x – 3.7477).

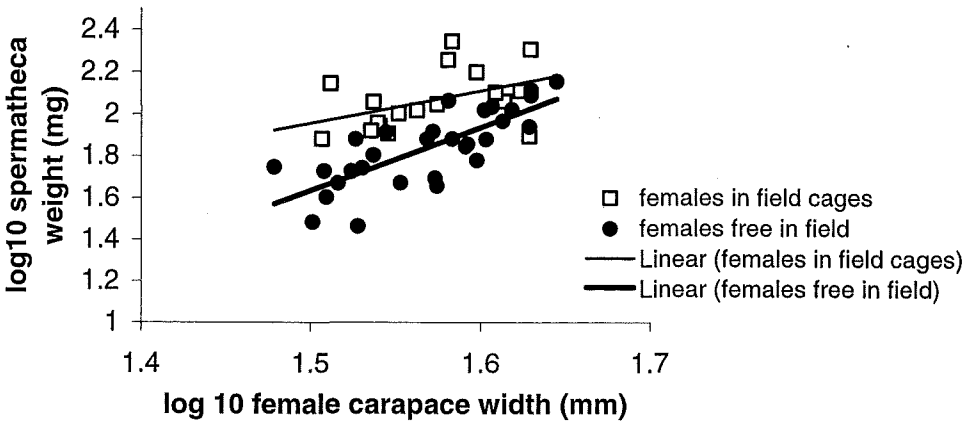


Figure 3.7 Spermatheca weight of ovigerous females from the field and field cages of *Hemigrapsus sexdentatus* in 2000. Linear regression equations, free field females (n = 30), y = 3.04x – 2.928, R^2 = 0.589; caged field females (n = 18), y = 1.5622x – 0.394, R^2 = 0.191.

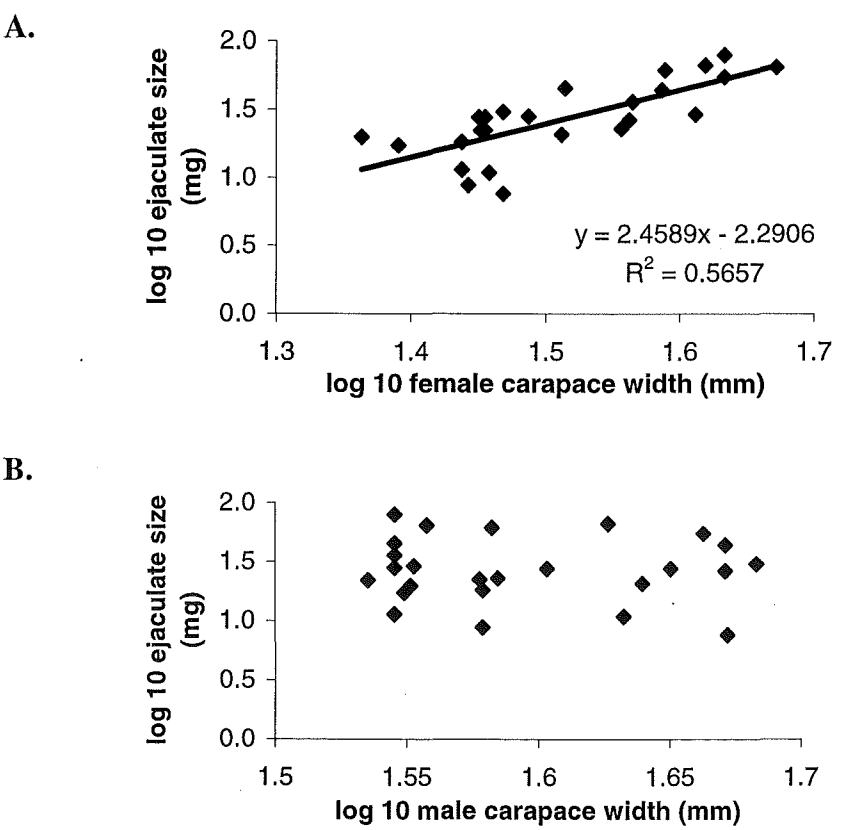


Figure 3.8 Relationship between ejaculate size and size of *Hemigrapsus sexdentatus*. A. Size of ejaculate received by females with empty spermathecae. B. Size of ejaculates transferred by males to females with empty spermathecae.

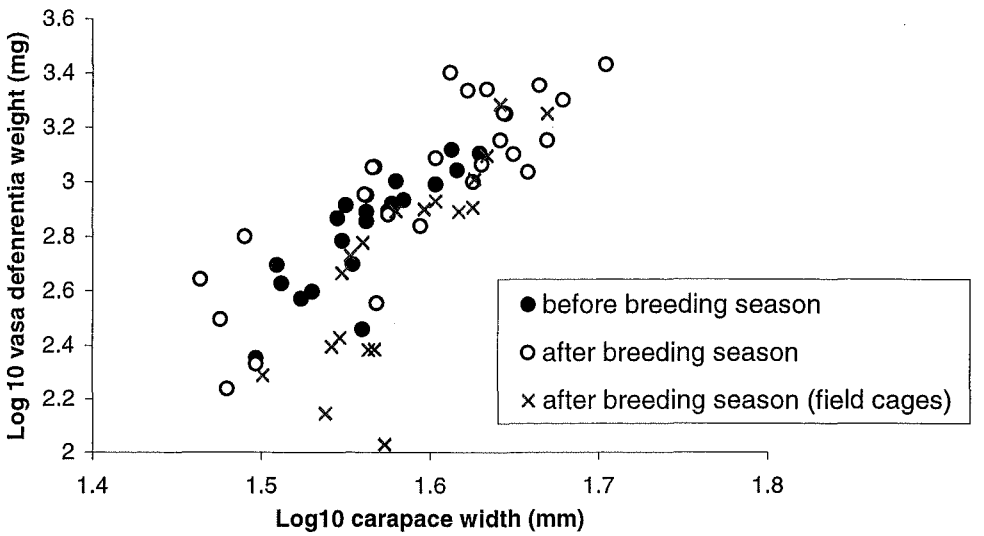


Figure 3.9 Weight of vasa deferentia of male *Hemigrapsus sexdentatus* before and after the mating season from a field population and after the mating season from males held in field cages with females.

4 Reproductive strategies of the intertidal crab *Hemigrapsus crenulatus* (Brachyura: Grapsidae) under different operational sex ratios and its consequences on male-male and sperm competition

Abstract - The reproductive behaviour of *H. crenulatus* was studied to investigate the fertilisation window of females and the impact of the operational sex ratio (OSR) on male-male and sperm competition. *Hemigrapsus crenulatus* had an asynchronous mating season over a period of about 5 months, during which females mated and oviposited multiple times. Males initiated pair formation, mated with and guarded receptive females until oviposition unless disturbed or replaced by another male. Under laboratory conditions, females were receptive during the intermoult, on the few days prior to oviposition. Females, which were completely or temporarily isolated from males stayed receptive on average for 11.9 d and 8.6 d, respectively, which was significantly longer than females housed continuously with males (mean receptive duration of 2.0 d to 4.1 d, depending on the sex ratio). This suggests that females are able to control their receptive duration (fertilisation window) according to the presence of males. However, when males were continuously present, the OSR had no effect on the duration of female receptivity. Most isolated females eventually oviposited and fertilised their ova, presumably with sperm stored in the spermatheca from the previous mating season. However, if isolated females delayed first mating and oviposition for longer than two weeks, the probability of reproductive success decreased drastically. The number of matings was positively correlated with the duration of the receptive period of the female and was influenced by the OSR and the temporary or constant presence of males. When several males were competing for one or two females (i.e., female to male OSR of 1:3 and 2:2), females mated almost twice as often than when only one male was present (i.e., female to male OSR of 2:1 and 1:1). In a more male-biased OSR, male-male competition therefore increased the number of matings per female and hence sperm competition within the female spermathecae. Furthermore, large males were significantly more successful in the number of matings than medium or small males because larger males were better at gaining access to a female, and guarding and defending them against smaller males. However, medium and small males frequently managed to gain brief access and mated with females. Consequently, depending on the way sperm is stored in the female spermatheca (i.e., in layers or mixed), small, medium, and large males might fertilise some of the ova. *Hemigrapsus crenulatus* was found to be infested by an internal parasitic isopod, *Portunion*

sp. (Entoniscidae). This parasite castrated the female host, preventing them from becoming receptive, and excluded them from any reproductive activities. In contrast, the parasite did not cause any noticeable morphological or behavioural alterations in its male host. Furthermore, during male-male competition parasitised males were equally successful as unparasitised males. In summary, females appear to be able to control their receptive duration according to the presence of males. Furthermore, male-male competition and sperm competition increased with a more male-biased OSR and caused higher mating frequencies for females. These observations demonstrate how such relationships can have a major effect on the outcome of sexual selection.

4.1 Introduction

According to Darwin (1871), sexual selection arises from differences in reproductive success caused by competition over mates, and is the main selective pressure responsible for differences between the sexes in size, shape, colouration, and behaviour. In crustaceans, for example, male crabs are often larger and have larger chelipeds than females, which is thought to be important for male success in male-male competition (e.g., Jivoff 1997b; Sainte-Marie et al. 1999). Such competition can be intensified through a biased sex ratio, particularly the operational sex ratio (OSR). The OSR is the ratio of fertilisable females to sexually active males at any given time, and depends on the degree of spatial and temporal distribution of the limiting sex (Emlen & Oring 1977). A biased OSR can increase opportunities for sexual selection. A strongly male-biased sex ratio can lead to stronger sexual selection for male traits favoured in competition over mates. It would, for example, predict that larger males are relatively more successful in gaining access to mates. Also in many crabs male size seems to play an important role in male mating success (e.g., Berril & Arsenault 1982; Abele et al. 1986; Fukui 1995). In commercially important crustacean species, the OSR often becomes more female-biased due to male-selective fisheries, and concerns have been raised regarding the long-term effects on stock quantity and quality (e.g., Smith & Jamieson 1991). Females may be unable to fertilise their ova successfully during their often limited time of sexual receptivity, when males, particularly large males, are selectively removed and become rare within a fished area. For example, sperm depletion of large males and inadequate sperm supply by small males has been reported for the red king crab *Paralithodes camtschatica* (Paul & Paul 1990, 1997), and a study on spiny lobsters (*Panulirus argus* and *Jasus edwardsii*) suggests that female fecundity could become sperm-limited in heavily fished populations where large males are typically rare (MacDiarmid & Butler 1999).

Consequently an increasing number of studies have been carried out over recent years on the reproduction of Crustacea, including such area as reproductive behaviour, the fertilisation process within the reproductive organs of the female, and sperm competition (= the competition between the sperm from two or more males for the fertilisation of a given set of ova, see Parker 1970).

In addition, sexual selection by female choice, in particular cryptic female choice, has gained increased attention (see Eberhard 1996). Classic female choice refers generally to inter-sexual interactions prior to copulation, whereas cryptic female choice occurs after copulation and has been investigated mostly in insects and to a much lesser extent in Crustacea. Cryptic female choice is defined as a "female-controlled process or structure that selectively favours paternity by males with a particular trait over others that lack the trait when the female has copulated with both types" (Eberhard 1996). If the OSR changes, female mating strategies might in turn be influenced due to a changed set of options, e.g., the presence of fewer and smaller males. Could females then compensate for this, for example, by extending their receptive period to increase the probability of finding a mate? It is generally believed that crustacean females have no control over their receptivity, so that reproductive activities are determined by some external, abiotic factors, such as the lunar cycle, temperature, etc. (Seiple 1979; Flores & Negreiros-Fransozo 1998), or internal, hormonal and developmental factors (De Kleijn & Van Herp 1998), or a combination of both (Caubet et al. 1998). However, a few examples are known in which females appear to have some control over their fertilisation window and to alter it according to availability of males, e.g., some Amphipoda (Ward 1984; Borowsky 1988), Isopoda (Mocquard et al. 1976; Shuster 1989), and Decapoda (Cowan & Atema 1990; Snyder et al. 1992; Henmi & Murai 1999).

Hemigrapsus crenulatus is a common intertidal crab in New Zealand and occurs in sheltered marine to estuarine habitats, where it burrows into mud or hides under stones (McLay 1988). The general reproductive biology of *H. crenulatus* in Canterbury, New Zealand has been investigated by Clark (1987), who found ovigerous females from June to January / February and suggested that females have two broods during this time. However, only anecdotal observations exist on the reproductive behaviour of this species (Yaldwyn 1966).

Crustacean reproductive behaviour and reproduction in general can also be affected by parasitism (e.g., *Sacculina granifera*; Shields & Woods 1993). *Hemigrapsus crenulatus* is frequently parasitised by the internal parasite *Portunion* sp. (Isopoda, Entoniscidae) (up to 32%, see Chapter 6). Species of the genus *Portunion* have been reported as parasitic castrators, particularly with respect to the female host (e.g., Kuris et al. 1980). *Portunion* sp. lives in the

hemocoel of the crab. Adult female *Portunion* sp. bear little morphological resemblance to free-living isopods and dwarf males live in and around the female's brood pouch (marsupium). The larvae are released into the water through a pore that connects the female parasite with the thin cuticle of the host gill chamber (Caullery 1952). Although most of the entoniscid isopods were described many years ago (e.g., Kossman 1881, Giard and Bonnier 1886; Drach 1941; Shiino 1942; Muscatine 1956), there are still only few studies available on host-parasite interactions (e.g., Atkins 1933; Veillet 1945; Kuris et al. 1980), and no studies have been carried out on the reproductive behaviour of parasitised crabs. Because *Portunion* sp. castrates particularly females, this parasite could have a strong influence on the OSR and hence intensify sexual selection. Among males, the parasite might disadvantage its host during male-male competition.

In this study, I examined the reproductive behaviour of *H. crenulatus* with emphasis on factors influencing the fertilisation window of females. Following the ideas of sexual selection and how it is influenced by the OSR, I wanted to examine whether female *H. crenulatus* have any control over the duration of their receptivity. For example, do females adjust the receptive period according to the sex ratio, level of male-male competition, or mating frequency to ensure the optimal, successful fertilisation of their ova? Furthermore, I wanted to investigate the effect of the operational sex ratio and male and female size on mating behaviour with regard to mating frequency, male-male competition, and sperm competition. In addition, I wanted to examine whether the internal parasite *Portunion* sp. affects the mating behaviour of its host, *H. crenulatus*.

4.2 Materials and Methods

Hemigrapsus crenulatus were collected monthly from June 1998 to May 1999 using baited net traps at Governors Bay in Lyttelton Harbour (43° 38′ S, 172° 39′ E), Canterbury, South Island, New Zealand. A total of 2300 crabs (1046 females and 1254 males) were examined (June, 197 females / 190 males; July, 122 / 114; August, 98 / 85; September, 65 / 73; October, 38 / 117; November, 48 / 107; December, 122 / 140; January, 45 / 69; February, 77 / 99; March, 100 / 98; April, 84 / 100; May, 50 / 62). Mainly larger crabs were caught with this method (i.e., larger than 10 mm carapace width; range, 6 - 37 mm; smallest ovigerous female, 10.3 mm). In addition, 57 females were collected by hand from the Avon-Heathcote estuary (43° 33′ S, 172° 44′ E), Canterbury, in May 1998 and some of these were also used in mating trials. Crabs were taken to the laboratory where they were measured (carapace width (CW), using a Mitutoyo

digital callipers to the nearest 0.1 mm), sexed (using the relative abdomen width; females have a wider/broader abdomen than males), and the reproductive stage of the female was determined (i.e., ovigerous or not, and whether the gonopore opercula were mobile, see below). Crabs were held under a 12 h light-dark cycle in tanks with circulating seawater of 12 - 15°C in the laboratory. Males and females were kept in separate group tanks and fed opened blue mussels (*Mytilus edulis*) three times a week unless otherwise stated.

To assess female receptivity, which is a prerequisite for assessing the time of mating, the gonopore opercula of all captive, mature, unparasitised (see below) females (CW > 10 mm) were probed daily just before and during the mating season (June to October 1998) and on the day of collection outside the breeding season (November 1998 to May 1999). To determine operculum mobility, the abdomen was slightly lifted and one of the two opercula was probed carefully with fine forceps under a binocular microscope at 160×. When the opercula were mobile, and could be pushed inwards like a trapdoor, females were considered receptive. If the opercula were immobile and could not be moved, females were counted as being non-receptive. Ovigerous females, had mobile opercula up to two days after oviposition, but were assumed to be non-receptive, as mating did usually not occur once a female had laid eggs (80 ovigerous females were tested in the laboratory and only one mated once; personal observation). Females were individually marked on their first receptive day with small, coloured, numbered bee tags (round plastic discs of 3 mm diameter, glued to the carapace with cyanoacrynalate glue).

General mating behaviour and female receptivity

To examine the mating behaviour of individual females and their receptivity on a daily basis, short-term experiments were carried out each day during the receptive period of females (males temporarily present on each receptive day). One receptive female, a small male (13.9 - 25.9 mm CW) and a large male (19.7 - 35.4 mm CW) were placed in a tank. In each set-up the large males were always larger than the female, and the mean size difference between small and large males was 5.5 mm. The glass tank (25 cm long × 25 cm wide × 25 cm high) was filled with seawater (12 - 15°C) to a depth of 15 cm, contained one rock for shelter, and was kept at ambient room temperature (about 19°C). The crabs were observed for a minimum of one hour each day. If a female mated within this first hour, the male she mated with was removed after copulation. The remaining two crabs were then observed for another hour to see whether the second male mated with the female. Males that mated were not used in the following three days to reduce the potential for sperm depletion and a decrease in male sexual activity. Notes were taken on mating

behaviour, frequency, and duration. A total of 30 females were observed each day during their receptive period, resulting in 227 short-term trials with 143 observed matings.

To investigate whether females mate prior to the second oviposition, 22 females that had mated for their first batch of eggs and were receptive for the second time in the breeding season, were placed together with two males for three hours. Their behaviour was observed and any matings were recorded.

To investigate whether females mate after moulting, 20 freshly moulted females (i.e., no later than 24 hours after moulting) were placed together with two males in a water-filled glass tank (size and conditions as above) and their behaviour was observed for three hours.

To examine whether males and receptive females are equally likely to initiate pair formation, a male or a female was tethered and the other crab (of the opposite sex) was allowed to freely move around. The crab was tethered with rubber bands to an empty mussel shell, was placed in a glass tank (size and conditions as above), and allowed to 'adjust' there for 30 minutes before adding the other crab. The tank contained one similar sized empty mussel shell, to offer an alternative shelter place for the free crab. The behaviour of the crabs was observed for one hour and notes were taken whether the free crab made physical contact with the tethered crab.

To examine the duration of female receptivity when matings could not occur, 100 females were isolated from males and the duration of female receptivity and oviposition was monitored. Females were held in groups of 30 - 35 under a 12 h light-dark cycle in tanks with circulating seawater of 12 - 15°C.

To examine if mating can trigger oviposition, receptive females were isolated for between 2 - 20 days and then placed together with two males for one hour. The occurrence of matings and time of oviposition were monitored. A total of 102 experiments were carried out using the following numbers of replicates: 2 d isolated (n = 9), 5 d (n = 10), 7 d (n = 6), 8 - 9 d (n = 9), 10 d (n = 23), 11 - 12 d (n = 9), 15 d (n = 25), 17 - 20 d (n = 11).

Effects of the operational sex ratio on mating frequency and male-male competition

To examine the effects of the operational sex ratio on mating frequency and male-male competition, long-term experiments (males continuously present during each receptive day) were carried out using continuous video recording starting on the female's first receptive day and ending when she laid eggs. In addition, males and females of different sizes were used to investigate the effect of size on mating behaviour and mate competition. The following ratios and numbers of receptive females per mature males were used: two different-sized females (small, 17.5 - 23.2 mm CW; large, 12.9 - 16.8 mm CW) with one male (n = 10); one female with

one male (n = 10); two similar sized females with two different-sized males (small, 19.9 - 24.8 mm CW; large, 28.0 - 31.6 CW) (n = 5); one female with three different-sized males (small, 15.9 - 20.2 mm CW; medium, 21.7 - 26.2 mm CW; large, 26.6 - 36.7 mm CW; n = 11). For each trial, the crabs were placed in a glass tank (25 cm long x 25 cm wide x 25 cm high) that was filled with seawater to a depth of 15 cm, contained one rock for shelter, and was kept in a 15°C constant temperature room with a 12 hour light-dark cycle. The water was changed daily using plastic tubes for carefully draining and refilling to minimise disturbance of the crabs. One to two hours before the water-change, two opened blue mussels were placed in the tank. The mussels were removed just before the tank was refilled. The crabs were monitored using a video recorder on 24 h time-lapse mode (0.18 s video recording interval). Infrared light was used during night hours to allow video recording. The general mating behaviour, time of the day (day *vs.* night), frequency, and duration of matings were recorded. In addition, results from the short-term trials, where males were temporarily present on each receptive day (see above), were examined for aspects of male-male competition.

Effects of parasites on mating behaviour and reproduction

When female crabs were parasitised by mature stages of *Portunion* sp., the parasite could often be detected from the outside. The crabs were examined by lifting the abdomen slightly and investigating the colour and movement behind the soft tissue between the abdomen and thorax. When a crab was parasitised, the beige abdomen of the mature parasite could then usually be seen. Parasitised females were not used in mating trials as they did not develop mobile gonopore opercula and were therefore morphologically unable to mate. Furthermore, males did not try to mate with parasitised females in the laboratory (n = 40 parasitised females). In addition, such females usually had no or largely reduced ovaries. This method did not detect all parasites, in particular not the early parasite developmental stages. Therefore, all mature females that had not become receptive by the end of the mating season were dissected. All of these 'left-over', mature females were found to be parasitised.

The parasite could be seen less easily in male crabs because the abdomen of males is narrower and the area between the abdomen and thorax is smaller. Therefore, no attempt was made to determine the presence of absence of the parasite prior to the mating trials. Instead, male crabs were dissected after the long-term mating trials to determine the presence or absence of *Portunion* sp. and its possible effect on mating behaviour. The general mating behaviour and mating frequency of parasitised and unparasitised males were then compared.

Sperm storage and ejaculate size

To study the spermathecae of ovigerous females from the field, these females were dissected and their spermathecae examined and weighed. These ovigerous females were collected at the beginning of the breeding season and were most likely carrying the first batch of eggs of the current breeding season. To estimate mating activity in the field, the weight of the spermathecae were compared with those of ovigerous females with a known number of matings observed in the laboratory.

To estimate the amount of ejaculate transferred to empty spermathecae, the weight difference between spermathecae that were filled during one copulation and the calculated weight of the empty spermathecae of a female of a given size was calculated. Similarly, to estimate the size of the second ejaculate, the weight difference between spermathecae which were filled during two copulations and the calculated weight of the spermathecae after one mating of a given female size was calculated. The relationship between the empty spermathecae weight and its weight after one mating and female carapace width was determined using linear regression.

Before dissection crabs were killed by placing them in a freezer at -15°C for about 1 hour. The carapace and the upper internal organs were removed to expose the gonads. Spermathecae, two in each female, were examined and dissected out by a cut close to the gonopore. Spermathecae were considered full, when they were clearly visible as two large, round, fully filled 'balloons' when the overlying internal organs were removed. The fresh weight of the spermathecae (two combined from each female) was determined to the nearest 0.1 mg.

Terminology and statistical analyses

Females of *H. crenulatus* are referred to as being receptive when they have mobile gonopore opercula in the days prior to oviposition. The duration of female receptivity is defined as the time from the first day a female has mobile gonopore opercula up to the day she lays eggs, or if no oviposition occurred up to the day the opercula became immobile. 'Empty' spermathecae are defined as spermathecae of unmated females, meaning they have not mated in the current mating season. However, these 'empty' spermathecae may contain some sperm stored from the previous mating season. Mean values given are followed by the standard error of the mean. Data were analysed using SYSTAT 9.

4.3 Results

The mean sex ratio of *H. crenulatus* was 0.82 females to one male and was usually male-biased over the one year sampling period (range, 0.32 to 1.15). Ovigerous females were found from July to January with a peak of 67.7% in September (Fig. 4.1). All mature females collected in the field that were transferred to the laboratory became receptive during the breeding season, unless they were parasitised and castrated by the entoniscid isopod *Portunion* sp. Mating and oviposition occurred asynchronously within the population over several months. Females observed in the laboratory laid eggs up to three times during the breeding season. Although nearly 250 females were caught with baited traps during the main breeding season from August to November, no receptive females were collected. Based on observations made in the laboratory, this is most likely due to the fact that receptive females were guarded by males and prevented from foraging. Although not directly measured in the field as no receptive females were caught, the operational sex ratio in the field is likely to be highly male-biased because breeding occurs asynchronously with only a few receptive females available at any time (see also below).

General mating behaviour and female receptivity

The general mating behaviour of *H. crenulatus* was very similar to *Hemigrapsus sexdentatus* (see Chapter 3). Females with mobile gonopore opercula mated during the intermoult a few days prior to oviposition. Matings were commonly observed from the first receptive day onwards until oviposition. Only rarely were matings observed after oviposition (1 out of 80 ovigerous females mated), although the gonopore opercula remained mobile for another two to four days. Lacking any courtship behaviour, a male typically approached, grasped, and manoeuvred a receptive female with his chelipeds and legs to face him. The male moved under the female while pulling her, resulting in the typical female over male copulation position (Fig. 4.2a). The duration of copulation was on average 15.7 ± 0.6 min (range: 4 - 35). Pair formation took usually only a few minutes, but in a few cases took several hours when the female was not 'co-operating' and resisted the mating attempts by the male. Males mated with the same female repeatedly and guarded her constantly (Fig. 4.2b) until she laid eggs, unless he was disturbed or displaced by another male. Males also guarded the females until oviposition when no other male was present (e.g., in the OSR of 1:1 during the long-term trials). Females appeared inactive during pair formation and postcopulatory guarding. However, females usually tried to escape during attacks by intruding males. As females occasionally lost a limb or received other damage

to her exoskeleton during struggles between males, it appears that escaping from and avoiding male fights reduces the risk of injuries to the female. Overall, females did not display an overt mate choice, but rather mated with males of any size that pursued her.

The time of day (day versus night) had no significant effect on the mating frequency (paired t-test: $t = 1.118$, $df = 10$, $P = 0.290$; 11 replicates) in the trials where males were constantly present. Out of a total of 83 matings, 47 matings occurred during the day and 36 matings during the night.

Most females that were receptive for the second time in the breeding season re-mated prior to laying the second batch of eggs (18 out of 22). Therefore, although the spermathecae were filled with relatively 'fresh' sperm from the current breeding season (i.e., about two months old), and females were generally able to use stored sperm, they commonly re-mated. None of the newly moulted females mated, suggesting that they were not receptive after moulting.

In experiments investigating the initiation of pair formation using tethered crabs, 10 out of 20 free males approached tethered receptive females whereas significantly fewer free receptive females approached tethered males (only 3 out of 20) (Chi-square test: $\chi^2 = 5.58$, $df = 1$, $P = 0.018$). Free crabs typically grasped and pulled either the chelipeds or legs of the tethered crab. Although only few females approached tethered males, these observations reveal that receptive females do occasionally initiate pair formation, at least when they are not being approached by a male.

The receptive duration of females was significantly affected by the absence, temporary or continuous (with 4 different OSR) presence of males (ANOVA: $F_{5,175} = 38.97$, $P < 0.001$) (Fig. 4.3). For example, isolated females stayed receptive on average for 11.9 days, which was significantly longer than that of females that were temporarily together with males on each receptive day (receptive for 8.6 days) and females that were continuously together with males during the entire receptive period (receptive for 2.0 - 4.1 days, depending on the OSR) (Fig. 4.3). This indicates that isolated females extended their receptive period and increased the probability of mating. Females that were temporarily housed with males also had a significantly longer receptive period (on average over twice as long) than females that were continuously housed with males (Fig. 4.3). However, as these two groups of females had on average a similar number of matings, the prolonged duration of female receptivity is not likely to be caused by the lack of mating events as suggested for the isolated females, but might be related to the quality of male partners (see also 'last male to mate' below). The receptive duration of females continuously housed with males was not significantly affected by the sex ratio (Tukey test: in each case, $P \geq 0.794$) (Fig. 4.3).

The duration of mated female receptivity was independent of size. However, in isolated females, duration was positively correlated with size (i.e., the larger the female the longer the receptive duration) (linear regression: $R^2 = 0.096$, $F_{1,98} = 10.43$, $P = 0.002$) (Fig. 4.4). The number of matings was significantly correlated with the receptive duration in the short-term and long-term trials (males temporarily or continuously present, respectively) (Table 4.1). The longer females were receptive, the more often they mated.

All females from the short-term and long-term mating trials laid eggs, whereas only 76% of completely isolated females did. Also some females which were temporarily isolated for several days of their receptive period, before giving them chance to mate, did not lay eggs. The longer the duration of isolation of receptive females the less likely they were to mate and lay eggs (Fig. 4.5). Females isolated only at the beginning of the receptive period mostly mated and laid eggs some time later, but only half of the females isolated for the first two or more weeks of receptivity subsequently mated and laid eggs (Fig. 4.5). In the first week of isolation (i.e., 2 - 5 days isolated), 44.4% - 55.6% of the females that mated oviposited within 24 hrs, in the second week (i.e., 7 - 12 days isolated), between 84% and 100%, and in the third week (i.e., 15 - 20 days isolated) 33.3 - 84.6%, respectively. In the first week females did not lay eggs soon after mating. However, in the second week mating seemed to trigger oviposition. In the third week, mating and oviposition events were overall markedly reduced, but in those females that did mate this was often followed by oviposition within 24 hrs. These findings therefore suggest that isolated females can 'wait' for mating opportunities up to 12 days without markedly reducing their productivity, because most were still able to lay eggs. However, productivity decreased rapidly after 12 days, as very few females were able to lay eggs.

Females held in the laboratory appeared to start the breeding season earlier compared to females in the field. For example in the laboratory, receptive females occurred from mid-June onwards and by mid-July the cumulative percentage of receptive females (excluding juvenile and parasitised females) had reached 27.0%. By comparison, only 4.7% of females in the field (excluding juvenile and parasitised females) were found to be ovigerous by that time (Fig. 4.6). Similarly, more females held in the laboratory were reproductively 'active' in August and September than ovigerous females collected from the field during these months (Fig. 4.6). As no receptive females were collected by the method used, the exact number of reproductively 'active' females (which includes receptive and ovigerous females) in the field is not known, but rather the percentage of ovigerous females alone. The exact percentage of reproductively 'active' females on the day of collection in the field would therefore most likely be slightly higher, i.e., greater than 4.7% in July and 20.0% in August, because, at least some of the females

in the field are expected to be receptive. However, the large difference, for example in August, of 20% of ovigerous females in the field versus 68.6% reproductively 'active' females in the laboratory, is not likely to be explained by the lack of collecting receptive females in the field. This large difference is more likely caused by females starting to become receptive earlier in the laboratory compared to the field. Therefore, the number of receptive females from the laboratory cannot be used to accurately estimate the OSR in the field, but rather to indicate which months receptive females are likely to occur and that receptive females are likely to be rare at any one time in the field (causing a highly male-biased OSR).

Effects of the differences of the operational sex ratio on mating frequency and male-male competition

During their receptive period females mated between 1 and 11 times in the short-term trials and between 1 and 21 times in the long-term trials. The number of matings per receptive period was not correlated with female size (linear regression: short-term trials, $P = 0.269$; long-term trials, OSR of 1:1, $P = 0.921$; OSR of 2:2, $P = 0.491$; OSR of 1:3, $P = 0.693$; OSR of 2:1, $P = 0.317$). Mating frequency tended to decrease over the receptive period in the short- and long-term trials, except when two females were housed with one or two males (Fig. 4.7). It appears therefore that females are usually more 'attractive' to males at the beginning of the receptive period compared to the end. However, the general trend of decreasing mating frequency with time was not always found, e.g., when two females were together with one male (Fig. 4.7A) and in some cases the trend is only weakly significant (Fig. 4.7 C, D). The former can possibly be explained by the fact that the one male could mate only with one female at a time and the other female, although 'attractive', had to wait sometimes.

The mean number of matings per receptive period ranged from 3.1 to 8.7 in the short-term and long-term trials and was significantly affected by the operational sex ratio and by the temporary or continuous presence of males (ANOVA: $F_{4,76} = 4.56$, $P = 0.002$) (Fig. 4.8). In the long-term trials, females mated almost twice as often when several males were competing for one or two females (i.e., female to male OSR of 2:2 and 1:3) compared with a situation when only one male was housed with one or two females (i.e., female to male OSR of 2:1 and 1:1). In addition, females mated significantly more often when more than one male was present in the long-term trials compared to short-term trials (Fig. 4.8).

Interestingly, two females housed with one male had no fewer matings compared to a single female housed with one male, even though they had on average the same receptive periods (Fig. 4.8). This shows that in such a female-biased sex ratio males increased their own mating

frequency. The single male mated with both receptive females on 8 out of 17 days. The number of matings and the receptive duration of the two females (S, L) housed with one male was not significantly different (*t*-test: $t = 1.54$, $df = 16.7$, $P = 0.144$ and $t = 1.30$, $df = 12.3$, $P = 0.219$, respectively). However, in the first two days there was a tendency for larger females to be more likely to mate with the male compared to smaller females (large females, 100% mated on first day and 86% on second day; small females, 70% on first day, 75% on second day). This suggests that larger females sometimes have an advantage over smaller females when males are limited (i.e., in 'short supply'), and that males might sometimes 'prefer' larger females over smaller females, although they used any mating opportunities and regularly mated with both of them.

Similar results concerning mating frequency and OSR were obtained when the number of female matings was expressed as the daily mating frequency (matings per day) instead of the total frequency (matings per receptive period). The mean number of female matings per day was again significantly affected by the OSR and by the temporary or constant presence of males (ANOVA: $F_{4,76} = 19.55$, $P < 0.001$) (Fig. 4.9). In the long-term trials the mean number of female matings per day was higher if more than one male was present, and in addition was also always higher in all long-term trials compared to the short-term trials (Fig. 4. 9).

Therefore, although the receptive period of females in the long-term trials was significantly shorter compared to the females in the short-term trials, females in the long-term trials did not mate less often. This is most likely due to the fact that the crabs in the long-term trials had overall more time available for mating than crabs in the short-term trials, and because male-male competition increased female mating frequency.

Male-male competition for the females was high causing frequent attacks on pairs and fights between males, which resulted in large males being overall more successful. For example, larger males obtained significantly more matings. In the short-term trials, large males mated 85 times compared to 58 times for small males during 106 trials where mating occurred (Chi-square test: $\chi^2 = 10.20$, $df = 1$, $P = 0.001$). Large males were more often the only male to mate (48 out of 72) (Chi-square test: $\chi^2 = 16.00$, $df = 1$, $P < 0.001$) or the first male to mate (24 out of 34) (Chi-square test: $\chi^2 = 15.53$, $df = 1$, $P = 0.001$) on a receptive day of the female. When the first male was removed giving the second male an easier chance to mate, 33.3% of the small males and 38.2% of the large males subsequently mated with the female. Therefore, in both cases, there was a high probability of the female mating again with the second male. Similarly, in the long-term trials with an OSR of 1:3 (S, M, L), large males again obtained significantly more matings than medium and small males (ANOVA: relative male size, $F_{2,30} = 11.817$, $P < 0.001$;

Tukey test: L *vs.* M and L *vs.* S, P = 0.001) (Fig. 4.10). However, no differences in mating success were observed for medium and small males (Tukey test: $P = 0.998$). Large males mated also more often than small males in the OSR of 2:2 (S, L) (56 compared to 31 matings, respectively) (Chi-square test: $\chi^2 = 14.37$, $df = 1$, $P < 0.001$).

Larger males were more likely to be the last to mate with the female before she laid eggs. In the short-term trials, large males were the last to mate in 19 out of 30 cases (Chi-square test: $\chi^2 = 4.27$, $df = 1$, $P = 0.039$). Similarly, in the long-term trials with an OSR of 1:3 (S, M, L), large males were the last to mate in 8 out of 11 cases compared to 3 times for medium males and none for the small males. However, in the OSR of 2:2 (S, L), large and small males were equally likely to be the last to mate (5 out of 10 for both male sizes). It appears therefore, that most females preferentially laid eggs after mating with a larger male.

Effect of parasites on mating behaviour

About half the males (27 out of 53, 50.9%) used in the long-term mating trials were infested with the parasite *Portunion* sp. No differences were observed in the mating behaviour of parasitised and unparasitised males. In addition, the number of matings did not differ significantly between parasitised and unparasitised males (ANOVA: $R^2 = 0.568$, $F_{1,29} = 1.376$, $P = 0.250$; relative male size and parasitism as categorical variables). However, whether parasitised males transfer the same amount of viable sperm was not assessed. Parasitised females were castrated, never developed mobile gonopore opercula, and were therefore morphologically not able to mate.

Sperm storage and ejaculate size

Permanently isolated females were able to fertilise their ova presumably using stored sperm from the previous breeding season 6 - 8 months before. In addition, females must have been able to retain sperm throughout the moulting cycle, because mature females moulted either between consecutive broods or between the breeding seasons (personal observation).

The spermathecae weight increased significantly with female size when females were isolated (no matings) ($R^2 = 0.540$, $P < 0.001$), mated once ($R^2 = 0.296$, $P = 0.013$) or twice ($R^2 = 0.636$, $P = 0.006$) in the laboratory, or had been collected as ovigerous females (mated unknown times) in the field ($R^2 = 0.306$, $P < 0.001$) (Fig. 4.11). The number of matings had a significant effect on spermathecae weight (ANCOVA: $R^2 = 0.727$, $F_{5,118} = 35.06$, $P < 0.001$; matings grouped as: no matings, 1, 2, 3 - 6, 7 - 12, or unknown times in the field). All ovigerous females from the field had full spermathecae indicating they all mated during the current mating season. Furthermore, ovigerous females from the field had significantly heavier spermathecae than

isolated (unmated) females (Fisher's LSD: $P < 0.001$) and females that had mated once (Fisher's LSD: $P = 0.002$). However, their spermathecae were not significantly heavier than those of laboratory females that had mated twice or 3 - 6 times. By contrast, ovigerous females from the field had lighter spermathecae than females that mated 7 - 12 times (Fisher's LSD: $P = 0.002$). This suggests that females in the field mate multiple times, probably between two and six times (Fig. 4.11). Such multiple matings are certain to result in high sperm competition within the female spermatheca (see also sperm storage below).

No significant correlation was found between the size of females with 'empty' spermathecae (i.e., females that had not mated in current mating season) and the size of the first ejaculate they received (range: 7 to 164 µg) (linear regression: $R^2 = 0.105$, $F_{1,18} = 2.10$, $P = 0.164$) (Fig. 4.12A). The size of the estimated first and second ejaculates were also not significantly different (ANCOVA: $R^2 = 0.141$, $F_{1,25} = 0.24$, $P = 0.629$). Furthermore, no correlation was found between the size of males and the ejaculate they transferred to females with empty spermathecae (linear regression: $R^2 = 0.001$, $F_{1,18} = 0.013$, $P = 0.909$) (Fig. 4.12B). These results suggest, that males transferred a similar ejaculate irrespective of female size, similar ejaculates to females that had or had not mated previously, and that males of different sizes were able to transfer similar amounts of sperm.

4.4 Discussion

General mating behaviour and female receptivity

This is the first time that prolonged post-copulatory guarding has been documented for a grapsid crab (together with *H. sexdentatus*, see Chapter 3). Some pairs of *Gaetice depressus* have been reported to stay close to each other temporarily after copulation if it occurred close to a shelter and if the male was larger than the female (Fukui 1994). However, so far none of the grapsid species is known to remain with and guard the female until oviposition. This might be partially due to the sometimes anecdotal nature of the mating observations for some of the grapsid species. However, post-copulatory guarding by males is known from a range of other Crustacea, such as portunid and cancrid crabs (see Christy 1987). It has long been argued to be important in protecting newly moulted females from cannibalism and predation (when mating is associated with moulting) as well as reducing the risk of sperm competition and therefore ensuring paternity (e.g., Jivoff 1997b; a review of Birkhead & Møller 1998).

The initial mating behaviour of *H. crenulatus*, wherein males quickly approach and grasp females without any prior courtship display followed by a relatively short copulation of several minutes, was similar to that reported for other species of the genus and family (e.g., *H. nudus*, *H. oregonensis* (Knudsen 1964; Lindberg 1980), *Pachygrapsus transversus* (Abele et al. 1986)). Despite the overall similar mating behaviour within the family Grapsidae a range of male reproductive strategies can be found. Previously, Grapsidae where known to follow either a resource centered competition, where a refuge is defended by a male and used as a site for mating, or a encounter rate competition, where males follow a search/intercept strategy for receptive females, but neither defend females nor resources (see Christy 1987). However, this study shows that some grapsids also follow a female centered competition, where males search and defend females.

Female *H. crenulatus* were able to control the duration of their receptivity according to the absence or presence (temporarily or continuously) of males. When females had no or only limited access to males, they prolonged the fertilisation window, possibly to increase the probability of mating (i.e., when isolated) or possibly to encounter a higher quality male (e.g. larger male). However, females did not appear to control the length of the receptive duration to achieve a certain number of matings, as there was considerable variation in the number of matings, which was also independent of female size.

As female *H. crenulatus* control their receptive period, they also influence the OSR. A short receptive period promotes a more male biased OSR with increased male-male competition. This is likely to result in the female having the opportunity to mate with the better quality males, e.g., larger males (see below). In contrast, a long receptive period results in a less male-biased OSR. This provides the female with fewer options and increases the restriction she experiences while being constantly guarded by her mate over a longer period of time. Therefore, female *H. crenulatus* have a relatively short receptive period that gives her the advantage of mating with a high quality male and decreases the time of restriction by the guarding male, but is able to extend this if required (i.e., if males are rare).

Female *H. sexdentatus* under laboratory conditions were also observed to control their receptivity in a similar way to *H. crenulatus* (see Chapter 3). However, female *H. crenulatus* were capable of prolonging their receptive period relatively longer than *H. sexdentatus*. For example, the mean receptive period of isolated female *H. crenulatus* was 11.9 d, 8.6 d in the temporary presence of males, and 2.0 to 4.1 d, depending on the OSR, in the continuous presence of males. In contrast, the mean receptive duration of female *H. sexdentatus* was 6.3 d in the absence and 5.2 d in the presence of males. These differences could be due to the differences in

breeding periods and embryo and larval development of *H. crenulatus* and *H. sexdentatus*. *Hemigrapsus sexdentatus* is a synchronous breeder in which all females mate and lay eggs within three weeks (see Chapter 2). If female *H. sexdentatus* could extend their receptive period by several days, as does *H. crenulatus*, the breeding season of *H. sexdentatus* would be spread out over a month instead of being confined to around three weeks. This might result in the release of the slow-developing larvae later in the year when conditions could be more unfavourable, as the right timing of larval release is important in decapod crustaceans (Forward 1987). In contrast, *H. crenulatus* has an asynchronous breeding season over several months with two to three broods. Under these circumstances, even three-fold changes in the duration of female receptivity would still mostly occur within the normal breeding season. Furthermore, the release of larvae occurs already over several months and most changes would still be within the overall period of larval release. In addition, males of *H. sexdentatus* might also be adapted for a short, synchronised breeding season, and if females stayed receptive for longer, males might not be searching for them. In contrast, male *H. crenulatus* will be adapted for searching for receptive females over several months during the asynchronous breeding season, and if females extend their receptive period within the breeding season, they are likely to be found by a searching male.

Mating appeared to trigger oviposition in the second week of temporarily isolated female *H. crenulatus*, which presumably prolonged the receptive period to increase the probability of mating. However, if isolated females extended the receptive period for too long (more than 2 weeks), the mating probability decreased and females often failed to reproduce during that particular receptive period. Similarly, female *Gammarus palustris* have been reported to be able to delay copulation in the absence of males (Borowsky 1988). Usually, copulation in *G. palustris* occurs within minutes after moulting and oviposition about an hour after that. When male *G. palustris* were absent, copulation could be delayed for 42 ± 6 hours. Similar to the pattern of decreased mating probability with time found for *H. crenulatus*, female *G. palustris* were less and less likely to mate as time passed since moulting (Borowsky 1988). Furthermore, when copulation was delayed for more than six hours in *G. palustris*, fecundity was also significantly reduced (Borowsky 1988).

Completely isolated female *H. crenulatus* were more likely not to lay eggs (24%) than female *H. sexdentatus* (always laid eggs, see Chapters 2 and 3). It is not known, if and when previously isolated female *H. crenulatus* continue to reproduce, however, it is probably likely that females again become receptive a few weeks later and then reproduce, or they might moult and then reproduce. As *H. crenulatus* breeds several times during the breeding season, the

reproductive loss of one brood would be relatively small compared to the situation in *H. sexdentatus* which breeds only once per year. Therefore, when males are absent, *H. sexdentatus* is probably more likely than *H. crenulatus* to reproduce using old sperm from the previous mating season, as it would otherwise lose a whole year of reproduction.

The breeding season of female *H. crenulatus* held in the laboratory started earlier compared to the field, similar to observations made for *H. sexdentatus* (see Chapter 2). This is most likely due to different temperature (i.e., colder) and photo-period (i.e., shorter for *H. sexdentatus* and longer for *H. crenulatus*) regime in the laboratory compared to the field as both crabs begin breeding while temperatures decline and days become shorter. Spawning can be induced by changes in temperature and photoperiod in the shrimp, *Palaemonetes pugio* (Little 1968) and by changes in temperature in the blue crab *Callinectes sapidus* (Sulkin et al. 1976).

Effects of the operational sex ratio on mating frequency and male-male competition

The operational sex ratio had a profound effect on mating frequency and male-male competition in *H. crenulatus* as the number of female matings almost doubled when two or more males were competing and males aggressively fought over females. The high number of female matings in turn increased sperm competition within the female spermathecae. Interestingly, male *H. crenulatus* always guarded receptive females until oviposition irrespective whether or not another male was present.

Variation in the OSR leads to changes in reproductive behaviour in many other species, but not in all. For example, in a more male-biased OSR, male amphipods *Eogammarus oclairi* (Iribarne et al. 1995) and *Gammarus duebeni celticus* (Dick & Elwood 1996), isopods *Asellus aquaticus* and *A. meridianus* (Manning 1980), hermit crab *Pagurus middendorfii* (Wada et al. 1999), and swimming crab *Callinectes sapidus* (Jivoff 1997a) guard females longer, and male guppies *Poecilia reticulata* (Jirotkul 1999) increased the relative number of sneak mating attempts. In contrast, the duration of mate guarding was unaffected by OSR in the sand lizards *Lacerta agilis*. However, male size was important in sand lizards as larger males guarded females longer (Olsson et al. 1996).

Male size played an important factor in male-male competition in *H. crenulatus*. Larger males were generally more successful in gaining access to females, achieving a higher number of matings, and were more likely to be the last to mate (= most likely leading to higher probability to fertilise the ova, see below). For *H. crenulatus*, the dominance in mating success of large males appears to be mainly the outcome of male-male competition rather than the result of active female choice, as females mated essentially with all males that pursued her, irrespective of male

size. Therefore, there was no correlation between the sizes of males and females in mating pairs in the laboratory (data not shown). However, females might have selectively laid eggs after they mated with a larger male, as large males were often the last males to mate before the female oviposited.

Nevertheless, it has been shown that the relative size of males and females can be important for the reproductive behaviour of a species. For example, size related mating can be the result of energetically or morphological constraints to physically control (guard) a mate, the spatial or temporal distribution pattern of animals of a particular size, or active mate choice (Ridley & Thompson 1985; Crespi 1989). In *Gaetice depressus*, males approach and mate with females irrespective of the female's body size, whereas females approach usually only males larger than themselves (Fukui 1994).

Small and medium males occasionally mated with female *H. crenulatus* and might be able to fertilise some of the ova, depending on the pattern of sperm storage within the female spermathecae. Female *H. crenulatus* have ventral-type spermathecae, a type which has been reported to favour the last male's sperm during fertilisation (Diesel 1991; Koga et al. 1993; Urbani 1998). However, detailed investigations on sperm storage within the female spermathecae of *H. crenulatus* are necessary to establish a male's chances of paternity in relation to that of other males.

Other studies have found male size to be important in the outcome of male-male competition in several crustacean species, for example in the crayfish *Orconectes rusticus* (Snedden 1990) and *Austropotamobius pallipes* (Villanelli & Gherardi 1998)) and the crabs *Carcinus maenas* (Reid et al. 1994) and *Chionoecetes opilio* (Sainte-Marie et al. 1999). Furthermore, it has been shown for the spiny lobsters *Jasus edwardsii* and *Panulirus argus* that larger males are able to fertilise larger clutch sizes than smaller males (MacDiarmid & Butler 1999).

Effects of parasites on mating behaviour

The mating behaviour of male and female crabs was differentially affected by the internal parasite *Portunion* sp. Parasitised females were castrated, ignored by males, and did not mate. However, the parasite did not affect the mating behaviour of male crabs and male-male competition was not affected by the parasite. Larger males, although more likely to be parasitised (see Chapter 6), were usually more successful in the number of matings than medium and small males. This suggests that females did not distinguish between unparasitised and parasitised males and that male-male competition was not affected by the parasite. This internal

parasite therefore has a major impact at the population level, because it castrates the females, but does not affect the reproductive success of individual males. Furthermore, the parasite increases the already male-biased OSR as it causes female castration. Therefore, even fewer females will become receptive and available for mating at any given time during the mating season.

Sperm storage and ejaculate size

Female *H. crenulatus* were able to fertilise their brood with sperm stored from the previous breeding season, similar to other grapsid crabs, such as *H. sexdentatus* (see Chapter 3), *Gaetice depressus* (Fukui 1990), and *Hemigrapsus sanguinensis* (McDermott 1998).

The spermathecae weight of *H. crenulatus* increased with female size, a pattern also observed for other decapods such as *H. sexdentatus* (see Chapter 3), *Uca lactea* (Murai et al. 1987), *Callinectes sapidus* (Jivoff 1997b), and *Macrophthalmus hirtipes* (Jennings et al. 2000). As the amount of sperm increases with the size of ejaculates (for example in *Chionoecetes opilio* and *Callinectes sapidus* (Sainte-Marie & Lovrich 1994; Jivoff 1997b), it appears that heavier spermathecae of larger females contain more sperm than those of smaller females with lighter spermathecae.

It was estimated from the weight of the spermathecae that in the field female *H. crenulatus* mate two to six times, whereas female *H. sexdentatus* mate only once or twice. Consequently, males of *H. crenulatus* and *H. sexdentatus* appear to follow different strategies concerning the distribution of their sperm. Male *H. crenulatus*, which are typically confronted with a high mating frequency of the female and a long, asynchronous mating season, distribute similar-sized ejaculates, irrespective of female size. By contrast, male *H. sexdentatus*, which experience a comparatively lower risk of sperm competition during a short, synchronised mating season, invest larger ejaculates for larger females than for smaller females. In addition, the size of the first and second ejaculates transferred to a female by a male *H. crenulatus* were not significantly different, whereas the first was larger than the second for *H. sexdentatus* (see Chapter 3). However, for both species, male size did not affect the ejaculate size, meaning that small and large males transferred similar sized ejaculates. Furthermore, in both species post-copulatory guarding was employed as a means of reducing sperm competition and ensuring paternity.

Table 4.1 Relationship between receptive duration and mating frequency of female *Hemigrapsus crenulatus* under different operational sex ratios with males temporarily (short-term trials) and continuously present (long-term trials).

Operational sex ratio (number of receptive females per male)	Linear regression results for tests of receptive duration *vs.* mating frequency	Number of females
Males temporarily present		
1 : 2	$R^2 = 0.275$, $F_{1,28} = 10.61$, $P = 0.003$	30
Males continuously present		
1 : 1	$R^2 = 0.929$, $F_{1,8} = 104.49$, $P < 0.001$	10
2 : 2	$R^2 = 0.858$, $F_{1,8} = 48.46$, $P < 0.001$	10
1 : 3	$R^2 = 0.536$, $F_{1,9} = 10.41$, $P = 0.010$	11
2 : 1	$R^2 = 0.454$, $F_{1,18} = 14.96$, $P = 0.001$	20

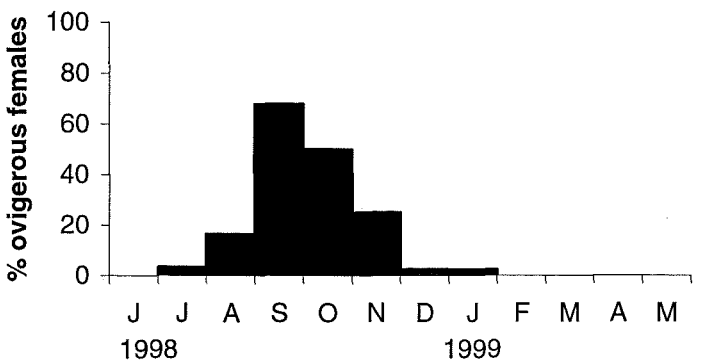


Figure 4.1 Percentage of ovigerous female *Hemigrapsus crenulatus* from June 1998 to May 1999.

Figure 4.2 *Hemigrapsus crenulatus*. A. Mating pair; B. Postcopulatory mate guarding (Note: Male holds female's leg with cheliped).

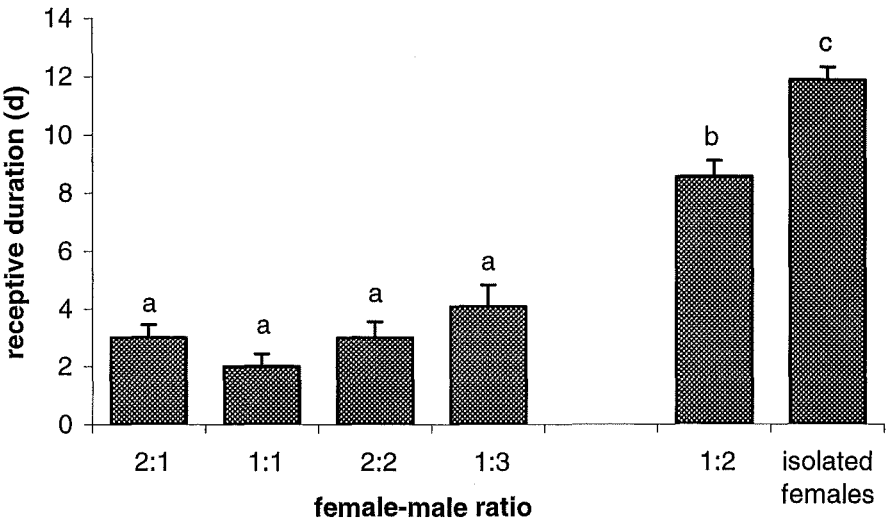


Figure 4.3 Receptive duration of female *Hemigrapsus crenulatus* under different operational sex ratios with males continuously (2:1, 1:1, 2:2, 1:3) or temporarily (1:2) present, and absent (isolated females). Letters above bars indicate significant results of Tukey test (2:1 *vs.* 1:2 and 2:1 *vs.* males absent, $P < 0.001$; 1:1 *vs.* 1:2 and 1:1 *vs.* males absent $P < 0.001$; 2:2 *vs.* 1:2, $P = 0.001$; 2:2 *vs.* males absent, $P < 0.001$; 1:3 *vs.* 1:2, $P = 0.009$, 1:3 *vs.* males absent, $P < 0.001$; 1:2 *vs.* males absent $P < 0.001$).

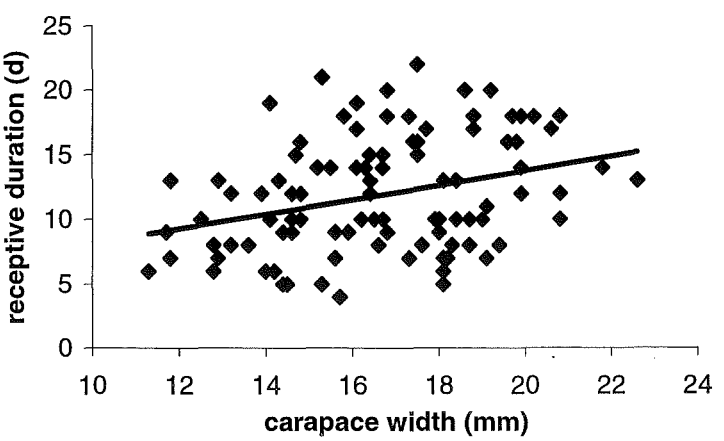


Figure 4.4 Receptive duration of isolated female *Hemigrapsus crenulatus* in relation to female size. Linear regression: $y = 0.5682x + 2.4803$, $R^2 = 0.096$.

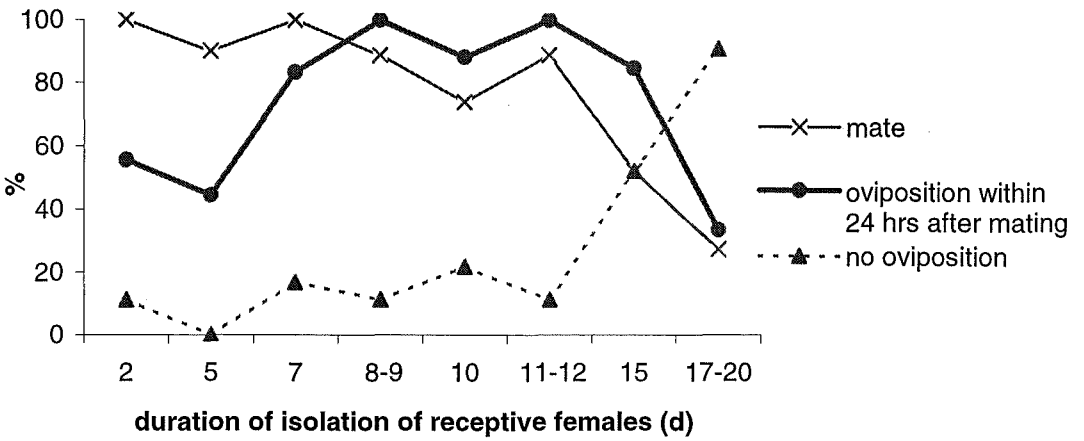


Figure 4.5 Mating and oviposition probabilities of previously isolated female *Hemigrapsus crenulatus*.

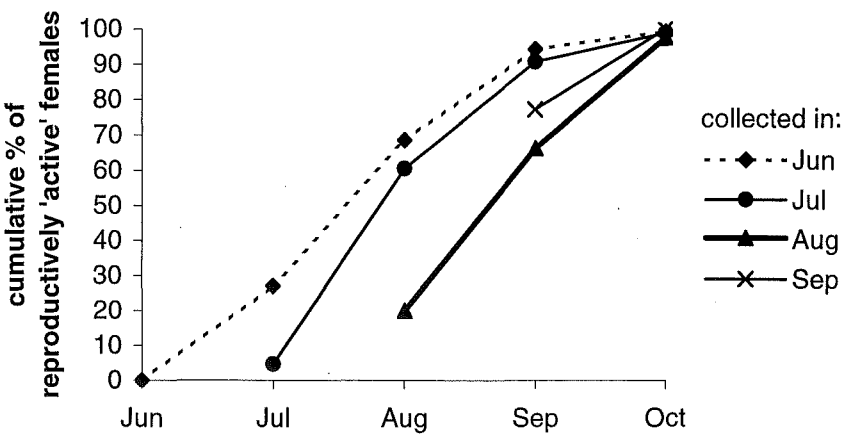


Figure 4.6 The cumulative percentage of reproductively 'active' female *Hemigrapsus crenulatus* during the first half of the breeding season in 1998. Note: this group of mature females includes ovigerous females collected from the field together with the cumulative percentage of receptive females observed in the laboratory, and excludes parasitised females.

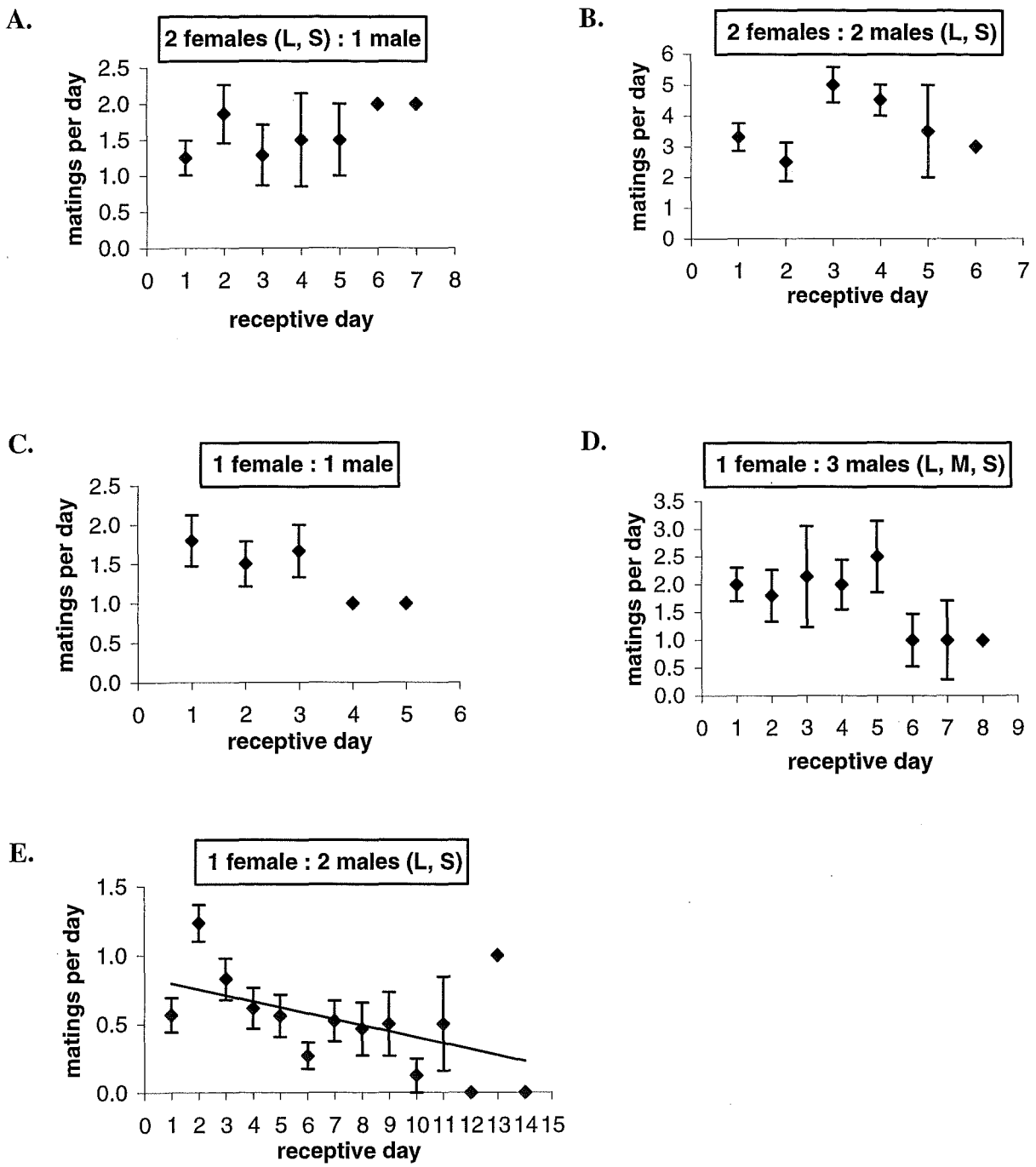


Figure 4.7 Mean number (± S.E.) of matings on each day of the receptive period of female *Hemigrapsus crenulatus* under different sex ratios and with males continuously (A – D) or temporarily (E) present. Relative crab sizes are indicated in parenthesis: L, large; M, medium; S, small. Linear regression equations and probabilities: A, y = 0.0982x + 1.2347, $R^2 = 0.432$, $F_{1,5} = 3.81$, $P = 0.109$; B, y = 0.0286x + 3.5333, $R^2 = 0.003$, $F_{1,4} = 0.01$, $P = 0.915$; C, y = -0.21x + 2.0233, $R^2 = 0.760$, $F_{1,3} = 9.52$, $P = 0.054$; D, y = -0.1658x + 2.4265, $R^2 = 0.462$, $F_{1,6} = 5.15$, $P = 0.064$; E, y = -0.0558x + 0.8777, $R^2 = 0.530$, $F_{1,12} = 13.54$, $P = 0.003$.

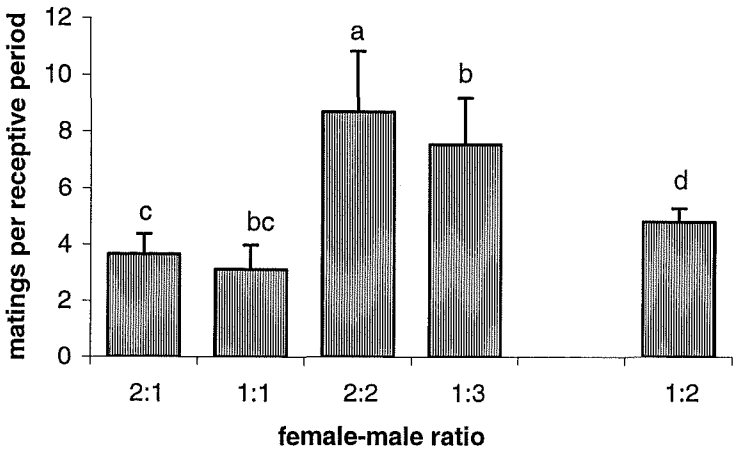


Figure 4.8 Mean number (± S.E.) of matings over the entire receptive period of female *Hemigrapsus crenulatus* under different operational sex ratios (F/M) with males continuously (2:1, 1:1, 2:2, 1:3) or temporarily (1:2) present. Letters above bars indicate significant results of Fishers LSD test (2:1 *vs.* 2:2, $P = 0.001$; 2:1 *vs.* 1:3, $P = 0.010$; 1:1 *vs.* 2:2, $P = 0.002$; 1:1 *vs.* 1:3, $P = 0.011$; 2:2 *vs.* 1:2, $P = 0.008$; 1:3 *vs.* 1:32 $P = 0.049$).

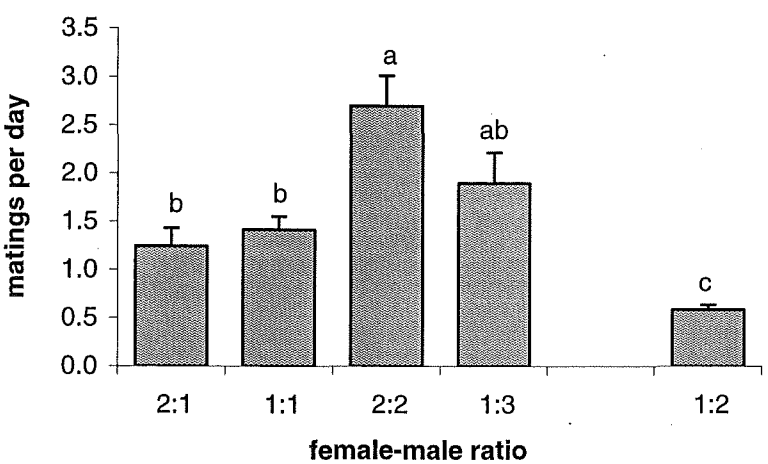


Figure 4.9 Mean number (± S.E.) of matings per receptive day of female *Hemigrapsus crenulatus* under different operational sex ratios (F/M) with males continuously (2:1, 1:1, 2:2, 1:3) or temporarily (1:2) present. Letters above bars indicate significant results of Fishers LSD test (2:1 *vs.* 2:2, $P < 0.001$; 2:1 *vs.* 1:3, $P = 0.013$; 2:1 *vs.* 1:2, $P = 0.002$; 1:1 *vs.* 2:2, $P < 0.001$; 1:1 *vs.* 1:2, $P = 0.002$; 2:2 *vs.* 1:3, $P = 0.014$; 2:2 *vs.* 1:2, $P < 0.001$; 1:3 *vs.* 1:2, $P < 0.001$).

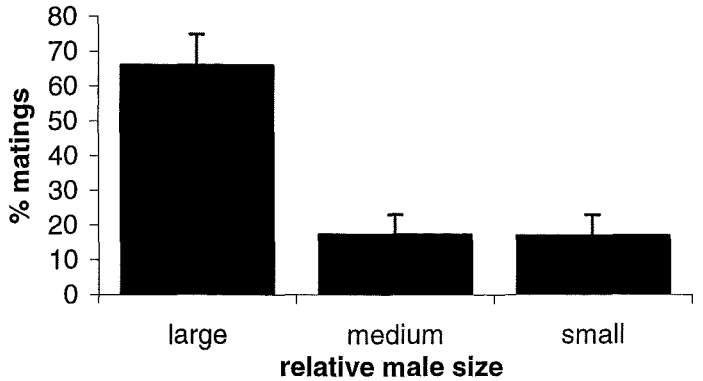


Figure 4.10 Relative mating success of large, medium and small males of *Hemigrapsus crenulatus* in the presence of one female in long-term trials (n = 11).

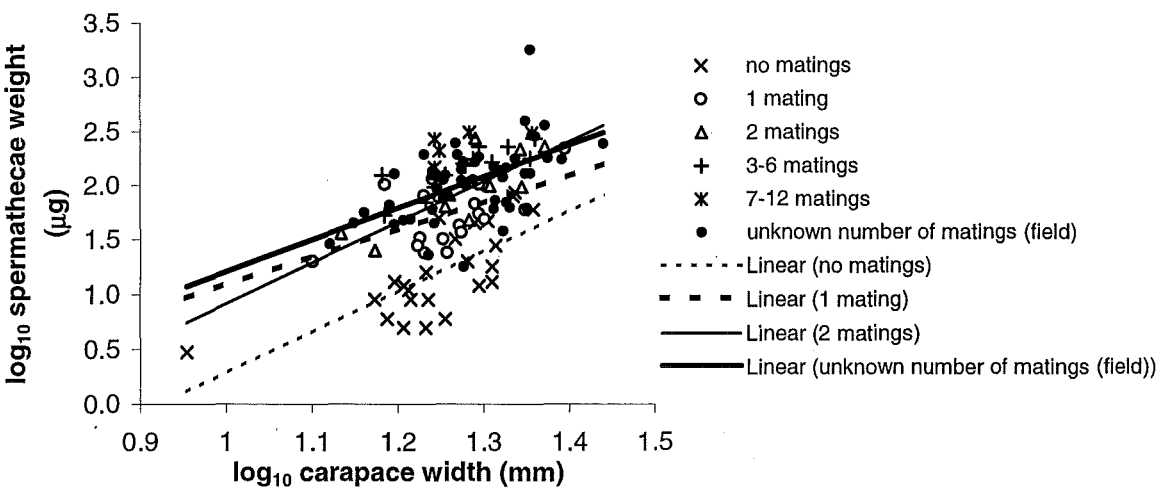


Figure 4.11 Spermathecae weight of ovigerous female *Hemigrapsus crenulatus* from the field and laboratory (M = number of matings; linear regression equations: no M (n = 25), y = 3.7019x – 3.4146; 1 M (n = 20), y = 2.5367x – 1.4507; 2 M (n = 10), y = 3.7446x – 2.8335; 3 - 6 M (n = 13); 7 - 12 M (n = 5); field (unknown number of matings (n = 52), y = 2.9227x – 1.7146).

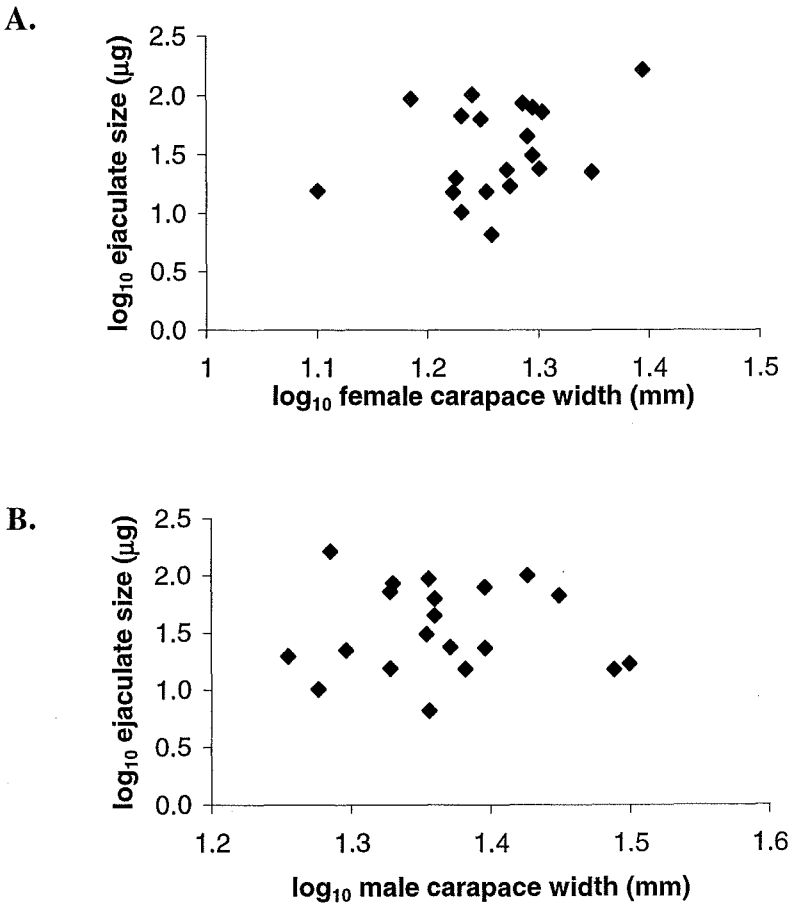


Figure 4.12 Relationship between ejaculate size and size of *Hemigrapsus crenulatus*. A. Size of first ejaculate received by females with empty spermathecae. B. Size of ejaculates transferred by males to females with empty spermathecae.

5 Comparative analysis of the mating strategies in grapsid crabs with special references to the intertidal crabs *Cyclograpsus lavauxi* and *Helice crassa* (Decapoda: Grapsidae) from New Zealand

Abstract - Field and laboratory observations were carried out on the reproductive behaviour of *Cyclograpsus lavauxi* and *Helice crassa* and the results compared with other Grapsidae with emphasis on New Zealand species. Mating in all species typically occurs during the intermoult and often coincides with the time of oviposition. Females of several species have been reported to mate multiple times, often in the few days prior to oviposition, leading to sperm competition within the female spermatheca. Females were found to be sexually receptive only in a short period before oviposition (e.g., *C. lavauxi*, *Hemigrapsus crenulatus*, *H. sexdentatus*), although some species were also receptive for about two weeks after oviposition (e.g., *Helice crassa*). The exact duration of female receptivity is unknown for most grapsid species. Female grapsid crabs exhibit a wide range of gonopore structures which either restrict female receptivity to certain times or allows them to mate at any time. In species with restricted female receptivity (e.g., *C. lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus*, *H. sexdentatus*), the operational sex ratio is typically highly male-biased, which was sometimes even more skewed due to parasitic castration of females. Male-male competition was found to be intense in the four New Zealand grapsids with frequent male-male interactions during which larger males were typically more successful in fights over females resulting in a greater number of matings. Post-copulatory guarding, which is assumed to reduce the risk of sperm competition and to ensure paternity, has been observed in a few grapsid species (*Hemigrapsus sexdentatus and H. crenulatus*) but not in others (e.g., *Cyclograpsus lavauxi* and *Helice crassa*). Overall, grapsid crabs employ a variety of reproductive strategies including direct competition between males for females with post-copulatory guarding, males securing resources as sites for mating, and males having only brief interceptions with receptive females. These differences in reproductive behaviour are discussed in the context of sexual selection and the ecological and environmental differences of the habitats that grapsid crabs occupy. Mating strategies of grapsid species can be very different even if they occupy the same habitat and the females have similar duration of sexual receptivity.

5.1 Introduction

The mating strategy of an animal is influenced by its 'ecological and behavioural potential to monopolise mates' (Emlen & Oring 1977). An asynchronous mating season, for example, will lead to a male-biased operational sex ratio and will intensify the potential for monopolisation and sexual selection (Emlen & Oring 1977). Social behaviour, including mating behaviour, has often been seen as an adaptation to the particular environment in which the animal lives (e.g., Seiple & Salmon 1982; Salmon 1983; Abele et al. 1986).

Several suggestions have been made concerning the general mating behaviour of crustaceans. For example, males of aquatic brachyurans are generally assumed to be attracted mainly by pheromones and have a prolonged courtship that is often followed by postcopulatory guarding (Salmon 1983). By contrast, terrestrial brachyurans are assumed to communicate mainly by visual and acoustic means and form short-lasting pairs without any pre- or postcopulatory associations (Hartnoll 1969; Salmon 1983). Although this general pattern often applies, semi-terrestrial crabs especially show a much more diverse pattern in their mating behaviour (see Christy 1987).

The mating strategies of grapsid crabs are likely to be influenced by the time and duration of female receptivity, the extent of male-male competition, sperm competition, and the habitat they occupy. Previously, observations of the reproductive behaviour of Grapsidae were often anecdotal with a limited number of detailed studies, despite the fact that this family is relatively common and abundant along most temperate and tropical shores and estuaries (e.g., Griffin 1971; Burggren & McMahon 1988; Fukui 1988). Recent studies relating to grapsid reproduction were concerned with general reproductive biology (e.g., Flores & Negreiros-Fransozo 1998; McDermott 1998), mating behaviour (e.g., Abele et al. 1986; Fukui 1993, 1994, 1995), reproductive structures, and sperm storage (e.g., Anilkumar et al. 1996, 1999; López Greco et al. 1999).

Crustacean reproductive behaviour and reproduction can also be affected by parasitic castrators (Baudoin 1975). The crabs *Cyclograpsus lavauxi* and *Helice crassa* are frequently parasitised by the internal parasite *Portunion* sp. (Isopoda, Entoniscidae) whereby parasite prevalence may exceed 40% (see Chapter 6). Species of the genus *Portunion* have been reported as parasitic castrators, particularly with respect to the female host (e.g., Kuris et al. 1980). However, no studies have been carried out on the reproductive behaviour of parasitised crabs. Among males, for example, the parasite could disadvantage its host during male-male competition.

The objectives of this study were to investigate the reproductive behaviour of two NZ grapsid crabs, the smooth shore crab *Cyclograpsus lavauxi* and the tunnelling mud crab *Helice crassa*. In particular, I was interested in the site of mating activity, the duration of female receptivity and mating frequency, and whether male size is important for male mating success. I also wanted to examine whether the internal parasite *Portunion* sp. affects the mating behaviour of its hosts, *C. lavauxi* and *Helice crassa*. Observations were carried out mainly in the laboratory with some additional observations made in the field.

Cyclograpsus lavauxi occurs on open coasts or sheltered bays in the upper half of the intertidal zone where it hides under stones (McLay 1988). Apart from the observation of one pair in copula in the field (Chilton & Bennett 1929), nothing is known about the mating behaviour of this species. However, it has been observed that reproduction mainly occurs during the summer months and that moulting mainly takes place after the breeding season (McLay 1988).

Helice crassa is endemic to New Zealand where it occurs on enclosed beaches, sheltered bays, lagoons and estuaries (Morton & Miller 1968). *Helice crassa* constructs burrows mainly above mid-tide level in well-drained sediment such as sand or mud, with some burrowing in salt meadows several metres above high tide (Nye 1977). A burrow is generally occupied by a single crab which forages usually within a 0.7 m radius around the entrance and defends the burrow against intruders (Beer 1959). The reproductive biology of *H. crassa* has been studied mainly with respect to seasonal changes in the occurrence of ovigerous females, including latitudinal variations, size at maturity, and the release of larvae (e.g., Nye 1977; Jones 1980; Jones & Simons 1983). Pairs mating on the mud surface have been observed by Beer (1959) and Nye (1977). However, it is not known if mating also occurs in burrows as in many ocypodid crabs (e.g., *Uca pugilator* (Christy 1982); *Scopimera globosa* (Koga et al. 1993); *Ilyoplax pusilla* (Henmi & Murai 1999).

Finally, I compared the reproductive behaviour of *Cyclograpsus* and *Helice* with the mating strategies of other grapsid species. It became apparent that grapsid crabs exhibit a wide range of reproductive strategies, from males either guarding or abandoning females after copulation, to defending resources used as sites for mating. Therefore, grapsid crabs have a similar diverse range of reproductive strategies as ocypodid crabs, although the latter appear to often have a more complex courtship display.

5.2 Materials and Methods

Collection of crabs

Crabs were collected between 1997 and 1999 at two sites in Canterbury in the South Island of New Zealand. *Cyclograpsus lavauxi* were collected by hand monthly from January to December 1998 during low tide from underneath rocks along the intertidal zone at Governors Bay in Lyttelton Harbour (43° 38′ S, 172° 39′ E). Mainly larger crabs were caught using this method (i.e., larger than 10 mm carapace width, range: 4.4 - 23.3 mm, smallest ovigerous female: 12.9 mm, n = 746 females and 904 males). Additional crabs (n = 513 females and 267 males) were collected from the same site for mating trials before and during the breeding season from October to December 1997.

Helice crassa were collected by hand every two months from October 1998 to August 1999 during low tide, using quadrats (0.3 m x 0.3 m) every two metres along a 40 m transect (perpendicular to the shoreline), from the Avon-Heathcote Estuary, Christchurch (43° 33′ S, 172° 44′ E)(n = 387 females and 438 males). Samples from seven additional 20 m transects were taken from September 1999 to February 2000 to obtain more information on the occurrence of receptive and ovigerous females and sex ratios during the mating season (n = 373 females and 468 males). Crabs smaller than 4 mm carapace width (CW) were not collected and only mature crabs (i.e., larger than 7.6 mm CW) were included in the data analysis and used in the mating trials. Additional mature crabs were collected from the same site for laboratory and field mating trials before and during the breeding season in August and September 1998 and 1999.

All collected *C. lavauxi* and *H. crassa* were taken to the laboratory, where they were measured (carapace width (CW), using Mitutoyo digital callipers to the nearest 0.1 mm), sexed (using the relative abdomen width; females have a wider/broader abdomen than males), and the reproductive stage of the female determined (i.e., ovigerous or not, and whether the gonopore opercula were mobile, see below). The egg developmental stage for *H. crassa* was determined using a stereo microscope and categorised using their main morphological features as criteria: stage 1, newly deposited with no cleavage and bright red yolk; stage 2, early cleavage and white tissue cap present; stage 3, chromatophores and limited dark brown yolk visible; stage 4, no yolk left, heart beat apparent, ready to hatch. Crabs were held under a 12 h light-dark cycle in tanks with circulating seawater of 12 - 15°C in the laboratory. Males and females were kept in separate group tanks and fed opened blue mussels (*Mytilus edulis*) three times a week unless otherwise stated.

To assess female receptivity, which is a prerequisite for assessing the time of mating, the gonopore opercula of all, mature, unparasitised (see below) females were probed daily for about four weeks before and during the mating season, and on the day of collection outside the breeding season. To determine operculum mobility, the abdomen was slightly lifted and one of the two opercula was probed carefully with fine forceps under a binocular microscope at 160×. When the opercula became mobile, and could be pushed inwards like a trapdoor, females had become receptive. Female *C. lavauxi* are referred to as being receptive when they have mobile gonopore opercula until oviposition (females do not mate after oviposition and the opercula become immobile within 24 hours). Female *H. crassa* are referred to as being receptive during the entire time their opercula are mobile including the days after oviposition (females continue to mate after oviposition and the opercula become immobile two to three weeks after oviposition). Females were individually marked on their first receptive day with small, coloured, numbered bee tags (round plastic discs of 3 mm diameter, glued to the carapace with cyanoacrynalat glue).

C. lavauxi: Mating behaviour, female receptivity, and sperm storage

To examine the mating behaviour of individual females and their receptivity on a daily basis, short-term experiments were carried out each day during the receptive period of females (males temporarily present on each receptive day). Groups of two to eight receptive females were placed in a tank with a group of mature males (14.5 - 26.0 mm CW) in a female to male sex ratio of 1 : 1 to 1 : 2. The plastic tank (45 cm long × 27 cm wide × 15 cm high) was filled with seawater (12 - 15°C) to a depth of 5 cm, contained several rocks for shelter, and was kept at ambient room temperature of about 19°C. The crabs were observed for three hours each day and notes were taken on mating behaviour, frequency, and duration. A total of 90 females were observed on each day of their receptive period, resulting in 469 receptive days with short-term trials and 51 observed matings. In addition, several receptive females were used for mating trials during some stage of their receptive cycle to obtain additional information on behaviour and spermathecal content (see also below). Males that mated in the short-term trials were not used in the following three days to avoid sperm depletion and a decrease in male sexual activity.

To examine the mating frequency of receptive females when males are constantly present, long-term experiments were carried over the entire receptive period of individual females using a video recorder on 24 h time-lapse mode (0.18 s video recording interval) in 1998 and 1999. Each receptive female was placed with three different sized males (CW small: 13.9 – 16.0 mm, medium: 17.1 – 19.1 mm, large: 19.5 - 22.4 mm, N = 13) in a glass tank (25 cm long × 25 cm wide × 25 cm high) that was filled with seawater up to a depth of 15 cm, contained one rock for

shelter, and was kept in a 15°C constant temperature room with a 12 hour light-dark cycle. Infrared light was used during night hours to allow video recording. The water was changed daily using plastic tubes for carefully draining and refilling to minimise disturbance of the crabs. One to two hours before the water change, two opened-up blue mussels were placed in the tank and these were removed just before the tanks were refilled. The general mating behaviour, time (day *vs.* night hours), frequency, and duration of matings were noted when video recordings were reviewed.

To study the spermathecae of ovigerous females from the field, freshly collected females were dissected and their spermathecae examined and measured. To estimate mating activity in the field, the weight of the spermathecae were compared with those of ovigerous females with a known number of matings observed in the laboratory. Before dissection, crabs were killed by placing them in a freezer at -15°C for about 1 hour. The carapace and the upper internal organs were removed to expose the gonads. The two spermathecae in each female were dissected by a cut close to the gonopore. Spermathecae were considered full, when they were visible as two large, round to elongate, fully filled 'balloons'. The weight of the two spermathecae combined was determined to the nearest 0.1 mg.

H. crassa: Mating behaviour and female receptivity

Laboratory observations

To examine the mating frequency of receptive females when males are constantly present, long-term experiments were carried out from the first receptive day of individual females until oviposition using a video recorder on 24 h time-lapse mode (0.18 s video recording interval) (n = 9). Seven of these females were further observed after oviposition for three to 16 days. Each receptive female was placed with three different sized males (CW small: 9.9 - 12.5 mm, medium: 13.0 - 15.1 mm, large: 15.3 - 20.0 mm) in a glass tank (35 cm long × 17 cm wide × 18 cm high). The set up of the tank, temperature and light conditions, and the water exchange and feeding regime was the same as for the long-term experiments of *Cyclograpsus*. The general mating behaviour, time, frequency, and duration of matings were recorded as for *Cyclograpsus*.

Long-term experiments were also carried out to examine whether burrows are used as sites for mating (11 trials with substrate) and to compare their mating behaviour with that of crabs in mating trials without substrate (above). Fine sand was added to glass tanks (sloping from 11 cm down to 2 cm), with the water level set at about 4 cm. Burrows dug by the crabs were not disturbed during the experiment (i.e., matings in burrows were only noted when pairs were not too far down into the burrow). The number of burrow matings is therefore a conservative

measure. Females either collected receptive from the field or from the laboratory were observed between one and eleven days prior to oviposition. The sex ratio, tank size, and light and temperature conditions were the same as above. Crabs were monitored with a video camera as above and notes on their behaviour were taken as above.

Field observations:

Crabs were observed during the breeding season in field enclosures to determine where matings occur (e.g., on the surface or underground or a combination of both) and whether males would court or guard females under natural conditions. Observations were carried out at low tide in September and October 1999, near my previous collection area in the Avon-Heathcote Estuary. Clear perspex enclosures (50 cm × 50 cm wide and 18 cm high) were placed onto the substrate in the upper shore area at low tide. Fifty-two non-ovigerous females, which were previously held in the laboratory, were marked on their carapace with dots and stripes of liquid paper, added to the enclosures, and observed every five minutes for one minute over one hour. Of these females, 38 had mobile opercula (i.e., receptive females; 10 replicates with 1, 11 replicates with 2, 2 replicates with 3 females per enclosure) and 14 had immobile opercula (i.e., non-receptive females; 7 x 2 females per enclosure). Notes were taken of their location (e.g., on surface or in burrow), and their behaviour such as walking, resting, digging, and mating. The behaviour of other crabs within the enclosure was noted if they interacted with the marked females. Subsequently all crabs within the enclosure were collected to determine the sex ratio (only mature crabs, i.e., larger than 7.6 mm CW were used in the analysis), and to expose pairs in burrows (= male guarding a female in a burrow or a pair mating). The number of burrow matings is a conservative measure as the burrows were inspected only at the end of the observation period and because females entering burrows before that could have also mated.

Effects of the internal parasite Portunion sp. on mating behaviour and reproduction of C. lavauxi and *H. crassa*

When female crabs were parasitised by mature stages of *Portunion* sp., the parasite could often be detected from the outside. The crabs were examined by lifting the abdomen slightly and investigating the colour and movement behind the soft tissue between the abdomen and thorax. When a crab was parasitised, the beige abdomen of the mature parasite could then usually be seen. Parasitised females were not used in mating trials as they did not develop mobile gonopore opercula and were therefore morphologically unable to mate. In addition, they usually had no or very reduced ovaries. This method did not detect all parasites, in particular the early parasite developmental stages. Therefore, all mature females that had not become receptive by the end of

the mating season were dissected. All of these 'left-over', mature females were found to be parasitised.

The parasite could less easily be seen in male crabs because the abdomen of males is narrower and the area between the abdomen and thorax is smaller. Therefore, no attempt was made to determine the presence of absence of the parasite prior to the mating trials. Instead, male crabs were dissected after the long-term mating trials to determine the presence or absence of *Portunion* sp. and its possible effect on mating behaviour. The general mating behaviour and mating frequency of parasitised and unparasitised males was then compared.

Female gonopore morphology

A range of grapsid species was investigated to study the gross morphology of female gonopores. The main purpose was to determine whether gonopore opercula were present, if they were fully developed or reduced, and if they were mobile. This would allow me to determine whether females are morphologically able to mate only at certain times (temporarily) or always (permanently). The gonopores were inspected under a stereo microscope at 160×. If opercula were present, they were probed carefully with fine forceps to determine whether they are mobile or immobile. In addition to females of *Cyclograpsus lavauxi* and *Helice crassa* that were examined on a daily basis as described above, I also examined *Hemigrapsus crenulatus* (Chapter 4) and *H. sexdentatus* (Chapter 3) before and during the mating season. Mature females of the following alcohol preserved species were also examined: 1 *Cyclograpsus insularum* from New Zealand, 1 *Eriocheir japonica* from Taiwan, 3 *Gaetice depressus* from Japan, 6 *Geosesarma peraccae* from Singapore, 1 *Leptograpsus variegatus* from New Zealand, 1 *Metopograpsus quadridentatus*, 1 ovigerous *M. frontalis*, and 1 *M. latifrons* from Singapore, 1 *Neosesarma gemmifrons* and 1 ovigerous *N. rectipectonatum* from Singapore, 3 *Plagusia chabrus* from New Zealand, 1 ovigerous and 1 non-ovigerous *Planes minutus* from New Zealand.

Terminology and statistical analyses

The family Grapsidae is currently undergoing a systematic revision that may result in the establishment of several families, e.g., Gecarcinidae, Glyptograpsidae, Grapsidae, Plagusiidae, Sesarmidae, and Varunidae (Schubart et al. 2000; Cuesta et al. 2001; Schubart et al. 2002). As this revision is still in progress, I refer to the family Grapsidae here in the broader sense, i.e. *sensu lato*, which includes all species formerly included in the family Grapsidae. The Grapsidae *s.l.* consists mostly of semi-terrestrial and intertidal species (e.g., *Cyclograpsus*, *Hemigrapsus*,

Helice) as well as some sub-tidal (e.g., *Plagusia*) and oceanic species (e.g., *Planes*, which live on floating drift materials or turtles) (Burggren & McMahon 1988; McLay 1988).

'Empty' spermathecae are defined as spermatheca of unmated females, meaning they have not mated in the current mating season. However, these 'empty' spermatheca may contain some sperm stored from the previous mating season. Mean values given are followed by the standard error of the mean. Data were analysed using SYSTAT 9.

5.3 Results

Cyclograpsus lavauxi: Mating season, mating behaviour and female receptivity

Ovigerous females were found during the Southern Hemisphere summer months from November to February (Fig. 5.1). Oviposition within the population was highly synchronised and occurred within about four weeks. Only a few receptive females were found in the field during the mating season from November to December, i.e., 12 out of 425 females in 1997 (0% - 7.1% females receptive at any one time; operational sex ratio (OSR, receptive female per male) of 0.010 - 0.053), and 4 out of 139 females in 1998 (2.2% - 3.2% females receptive, OSR of 0.018 - 0.020). Receptive females were not found during any other months of the year. However, as the mating season in 1998 started a few weeks later than in 1997, it is likely that some receptive females would have been found in January 1999, if monitoring had been continued. Nine ovigerous females were found with mobile gonopore opercula in November and December 1997 and one ovigerous female in December 1998. This indicated that these ovigerous females had just recently laid eggs, because the opercula become immobile within 24 hours after oviposition based on laboratory observations. The female to male sex ratio of *C. lavauxi* was male-biased in ten out of 12 months over the one-year sampling period (mean: 0.84, range: 0.62 to 1.11).

In the field, one pair was found in copula in a crevice between pebbles under a larger rock (approx. 20 cm wide), under which two other males were also found. The mating pair was transferred to a small, water filled container, in which they continued to mate for another 40 minutes.

In the laboratory, all mature females became receptive during the breeding season, unless they were parasitised and castrated by the entoniscid isopod *Portunion* sp. Females oviposited whether or not mating occurred.

In the laboratory (long-term trials), the mating behaviour of *C. lavauxi* was typically characterised by a male approaching a female and holding the female's carapace or pleopods

with his chelipeds for about an hour (53.4 ± 10.7 min, range: 5 - 187 min, n = 27), followed by a two hour copulation (116.1 ± 11.1 min, range 11 - 251 min, n = 27), which was then terminated by a sudden separation. Females often struggled against male mating attempts and escaped on several occasions. Sometimes a female lost a cheliped or walking leg during these struggles with males. In addition, females escaped when males were fighting over the female (see below). Overall, more matings occurred during the night (18 out of 27, 66.7%) than during the day (9 out of 27, 33.3%), but this difference was not significant (paired *t* test: $t_{12} = -1.559$, $P = 0.145$).

In the short-term trials, only half of the receptive females mated (46 out of 90, 51.1%), although all had several opportunities to mate during their receptive period. Of the females that mated, most mated once (41 out of 46, 89.1%) and the remainder mated twice. Therefore, females mated on average 0.6 ± 0.1 times during their receptive period in the short-term trials. The receptive period decreased significantly with female size when females did not mate (linear regression, $R^2 = 0.185$, $P = 0.004$, n = 44), but no correlation was found for females that mated. When all females were combined (mated and unmated) no correlation between receptivity and size was found (linear regression, $R^2 = 0.014$, $F_{1,88} = 1.269$, $P = 0.263$). The receptive duration of females that mated (6.2 ± 0.4 d, range: 2 - 12 d) was significantly longer than that of females that did not mate (4.3 ± 0.5 d, range: 2 – 15 d) (ANCOVA: $R^2 = 0.111$, $F_{1,87} = 9.487$, $P = 0.003$; carapace width covariate).

In the long-term trials, females were receptive for 6.3 ± 0.7 days (range: 2 - 10 days) and mated 2.1 ± 0.2 times (range: 1 - 3 times) during this time. Mating frequency tended to decrease over the receptive period (Fig. 5.2), however, no significant correlation was found (linear regression: $P = 0.287$). The receptive period was not correlated with female size (linear regression, $R^2 = 0.181$, $F_{1,11} = 0.974$, $P = 0.345$).

Females that were constantly together with males (i.e. in the long-term trials) mated significantly more often than females that were only temporarily together with males (i.e. in the short-term trials) (*t*-test: $t_{15.2} = 8.008$, $P < 0.001$). However, the duration of receptivity of females was not significantly different in the long- and short-term trials (ANCOVA: $R^2 = 0.018$, $F_{1,100} = 1.794$, $P = 0.183$; carapace width covariate). Neither in the short-term nor long-term trials was the number of matings correlated with female size (linear regression: $P = 0.526$ and $P = 0.179$, respectively) or the duration of receptivity (linear regression: $P = 0.954$ and $P = 0.162$, respectively).

Male size had a significant effect on the relative number of matings males obtained (ANOVA: $R^2 = 0.479$, $F_{2,36} = 16.548$, $P < 0.001$), with larger males obtaining on average 67.9% of the matings, medium males 25.6%, and small males only 6.4%. Larger males on average

obtained significantly more matings than medium sized males (Tukey: $P = 0.004$) and small males (Tukey: $P < 0.001$) (see also Fig. 5.9). However, no significant difference was found in the number of matings of medium and small males (Tukey: $P = 0.071$). In addition, larger males were more often the last male to mate with the female (7 out of 13 times) compared to medium (4 out of 13 times) and small males (2 out of 13 times).

One or two males frequently attacked pairs where the male was holding the female and sometimes when the pair was mating. These attacks mostly resulted in the female escaping and running away from the males (40 out of 61 attacks, 65.6%). In 18 out of 61 attacks (29.5%) in which a male retained control, it was the defending male that was able to hold on to and keep the female. Defenders were mainly large (45.9%) and medium (44.3%) sized males, and sometimes small males (9.8%). Attackers were mostly large males (53.8%), followed by medium (30.8%) and small males (15.4%). Only three take-overs were observed (4.9%), and these were all when a large male attacked a pair with a medium sized male. Therefore, an attacking male is not likely to win a receptive female from a pair directly unless he is large. However, the male might have a chance later on to capture the now single receptive female. Overall, male-male competition was intense with males frequently fighting over females.

Cyclograpsus lavauxi: Sperm storage

Females that did not mate were able to lay fertile eggs from which larvae hatched several weeks after oviposition (data not presented). These females presumably used stored sperm from the previous breeding season 10 - 12 months before. In addition, these females must have been able to retain sperm throughout the moulting cycle, as mature females typically moulted after the breeding season (personal observation).

The spermatheca weight increased significantly with female size when females did not mate (linear regression: $R^2 = 0.397$, $P < 0.001$) or mated once (linear regression: $R^2 = 0.775$, $P < 0.001$) in the laboratory, or had been collected as ovigerous females (mated unknown times) in the field (linear regression: $R^2 = 0.246$, $P = 0.001$) (Fig. 5.3). Furthermore, the spermatheca weight was significantly affected by the number of matings (i.e., no mating, one mating, or unknown number of matings in the field) (ANCOVA: $R^2 = 0.634$, $F_{2,121} = 47.516$, $P < 0.001$). All field ovigerous females had full spermathecae indicating that they had all mated at least once during the mating season. Field ovigerous females had significantly heavier spermathecae than females that did not mate (Fisher's LSD: $P < 0.001$), but their spermatheca weight was not significantly different compared to females that had mated once (Fisher's LSD: $P = 0.719$). Furthermore, the spermatheca weight of females that were known to have mated twice (n = 2,

Fig. 5.3) was also in the range observed for ovigerous field females. This indicates that all females in the field mate at least once and possibly more often, but that the number of "sperm" stored does not increase in proportion to the number of matings.

***Helice crassa*: Mating season, mating behaviour and female receptivity**

Female *Helice crassa* reproduce asynchronously over several months and ovigerous females were found in the field from early spring (August) to late summer (February) (Fig. 5.4). The majority of ovigerous females collected from transects had mobile gonopore opercula (133 out of 159, 83.6%), and of these, 86.5% had newly laid eggs at stage one. The other females with mobile opercula had eggs either in stage 2 (n = 9), stage 3 (n = 6), or stage 4 (n = 3). Ovigerous females with immobile opercula (n = 26) had also egg stages between one and four, but of those eggs 69.2% were in an advanced developed stage. Therefore, most females with newly deposited eggs were still receptive (i.e. having mobile gonopore opercula) and able to mate, whereas the females with more developed embryos were often not receptive, had immobile opercula, and therefore could not mate anymore.

Few receptive females without eggs (n = 38) were found in the transects and they occurred only during the breeding season. The female to male sex ratio was usually slightly male biased (mean: 0.88, range: 0.68 to 1.09) (Fig. 5.5). However, the operational sex ratio (OSR) for *H. crassa* was always highly male biased, ranging from 3 to 16 males per receptive female (OSR*, 0.063 – 0.309) and 13 to 67 males per non-ovigerous, receptive female (OSR**, 0.015 – 0.077) (Fig. 5.5). The operational sex ratio (OSR) was divided into OSR* (all receptive females (with and without eggs)) and OSR** (only receptive females without eggs) to distinguish between the two types of females that are both able to mate, but are assumed to use the sperm at different times. Receptive females without eggs have well developed ovaries with the ova ready to be oviposited and are therefore likely to use sperm within a few days (see laboratory observations below). These non-ovigerous, receptive females are therefore expected to be highly sought after by males, because their sperm might fertilise the ova in the near future. In contrast, ovigerous receptive females, which mate while carrying eggs, will first continue to brood their eggs before they might lay another batch of eggs some time later. Sperm of these females will therefore be stored for a longer period of time before it might be used (i.e., in the current or next breeding season). These ovigerous receptive females are therefore expected to be less 'interesting' for males compared to receptive non-ovigerous females, because paternity is less certain as the female might mate afterwards or the female might not survive before ovipositing again.

Mating behaviour observations in the laboratory

Mating trials without a fine sand substrate:

In the long-term trials without substrate, the mating behaviour of *H. crassa* was typically characterised by a male quickly approaching and grasping a female with his chelipeds, and within seconds manoeuvring himself underneath the female into the mating position. The pair remained in copula for 14.6 ± 0.5 minutes (range: 4 to 76 min, n = 219) after which they quickly separated. Sometimes, males guarded the females temporarily after mating (25 out of 219 matings, 11.4%), which lasted on average 142.5 ± 24.9 min (range: 13 to 436 min). However, males did not continue to guard females until females reached oviposition.

Females were receptive before oviposition for 12.4 ± 0.7 days (range: 10 - 15 days; n = 9) and mated 24.3 ± 4.6 times (range: 5 - 51 times; n = 9) during this time. Matings occurred during daytime (124 out of 219) and night-time (95 out of 219) and the number of matings were not significantly different during these times (paired t-test: t = 1.348, df = 8, P = 0.215). No correlation was found between receptive period and female size (linear regression, R^2 = 0.006, $F_{1,7}$ = 0.044, P = 0.840). The mean number of matings decreased significantly over the receptive period until oviposition (linear regression: $P < 0.001$) (Fig. 5.6). There was no correlation between the receptive duration and the number of matings until oviposition (linear regression: R^2 = 0.009, $F_{1,7}$ = 0.063, P = 0.809). Some females were observed for several days after oviposition (but not until the end of the receptive period when the opercula become immobile again). Females mated more often before oviposition than after (Fig. 5.7). Male size had a significant effect on the relative number of matings (ANOVA: R^2 = 0.665, $F_{2,24}$ = 23.871, $P <$ 0.001), with larger males obtaining on average 59.8% of the matings, medium males 27.8%, and small males only 12.4%. Larger males obtained on average significantly more matings than medium sized (Tukey: P = 0.002) and small males (Tukey: $P < 0.001$). Furthermore, medium sized males obtained significantly more matings than small males (Tukey: P = 0.018). In addition, larger males were more often the last male to mate with the female (6 out of 9) compared to medium (2 out of 9) and small males (1 out of 9).

Males often attacked pairs when the male was either holding (82 out of 136 attacks) or mating (54 out of 136 attacks) with the female. Attackers were mostly medium sized males (60.3%), followed by large (31.6%) and small males (8.1%). Defenders were mainly large (61.8%), followed by medium (30.1%) and small males (8.1%). The majority of these attacks were successfully repelled by the male of the pair (58.8%), especially when they occurred during mating. However, in 33.8% of the attacks, the pair was separated and the female ran away and escaped both males. Take-overs occurred only in 7.4 % of the attacks (10 out of 136) and

happened only when the attacker was larger than the defender. Therefore, once a male has gained access to a female he is likely to keep control of her especially when he is large. Furthermore, an attacking male is not likely to directly take over a receptive female, but has a chance to catch her later. Overall, male-male competition was intense with males frequently fighting over females.

Mating trials with a fine sand substrate:

In the trials where a substrate was provided for burrowing, crabs made burrows but were visible most of the time wandering around, feeding, or resting on the surface. The overall mating behaviour was similar to that described above in trials without substrate. Matings occurred mainly on the surface (76 out of 84 matings, 90.5%). In addition, four matings occurred at the burrow entrance of the males (4.8%) and four further down in burrows of the males (4.8%). Typically only one crab occupied a burrow at any time, and it is therefore not likely that many more matings occurred in the burrows. Pairs mated for 12.0 ± 0.5 minutes (range: 3 - 25 min, n = 84), after which they typically separated immediately afterwards (79.8%). Some males guarded the females after mating either on the surface (n = 14), at the burrow entrance (n = 1), or in the burrow (n = 2) for an average of 204.4 ± 56.9 min (range: 13 - 778 min). All pairs separated eventually independent of where the matings had occurred (i.e., no post-copulatory guarding until oviposition). Therefore, a particular burrow was not used for mating followed by oviposition. Twenty-one pairs that mated on the surface were attacked by another male. Mostly, the pair continued to mate (n = 13), however six pairs were forced to separate and the attacker managed twice to take over the female and to mate with her. None of the pairs that mated at the burrow entrance or inside the burrows were disrupted. Females were observed to lay eggs while on the surface as well as being ovigerous after leaving a burrow, apparently after laying eggs inside in the burrow.

Overall, 54 attacks on pairs (either pairs mating or male holding a female) were observed. Attackers were mostly medium sized males (40.4%), followed by large (33.3%) and small males (26.3%). Defenders were mainly large males (54.4%), followed by small (24.6%) and medium (21.1%) males. The majority of these attacks were successfully repelled by the male of the pair (59.6%), which stayed with the female. However, in 28.1% of the attacks, the pair was separated, and the female ran away and escaped both males. In seven cases (12.3%) the attacking male took over the female, and all of those males were larger than the defending male. The average number of attacks per day was similar in the trials with (1.1 attack per day, range: 0 - 2.5) and without (1.2 attack per day, range: 0.1 - 4.3) substrate.

Similar to the long-term trials without substrate, male size had a significant effect on male mating success (ANOVA: $R^2 = 0.485$, $F_{2,30} = 14.135$, $P < 0.001$). Larger males were again significantly more successful in the average relative number of matings (63.6%) compared to medium (18.0%) (Tukey: $P < 0.001$) and small (18.4%) males (Tukey: $P < 0.001$). The time of day (day *vs.* night) appeared to have an effect on the number of matings with a trend for more matings to occur at night (31 day matings, 53 night matings), although this was only marginally significant (paired *t* test: $t = -2.21$, $df = 10$, $P = 0.051$).

Mating observations in the field

The behaviour of 52 marked females was observed in field enclosures which contained a total of 455 mature crabs (233 females, 222 males). Marked females walked around, explored burrows, dug new or extended empty burrows, or rested within the enclosure, along the enclosure wall, or in the corner of the enclosure. The naturally occurring crabs within the enclosure seldom interacted with the added females. On two occasions a male ran quickly to a wandering, marked, receptive female and grasped her with his chelipeds. They mated within a few seconds on the surface for 6 and 8 minutes and separated immediately afterwards. No courtship or postcopulatory guarding behaviour was observed. A few times a burrow holder chased a marked female briefly when it had entered an occupied burrow or came close to a burrow entrance where the burrow holder was feeding. At the end of the observation period all crabs were dug up and four marked receptive females were found paired with a male in a burrow. In two cases the pair was mating and in the others, the marked receptive female and the male were very close together, as if they had just separated or were interrupted from mating. If these four burrow pairs are all assumed to have mated as did the two on the surface, then 15.8% of the receptive females (6 out of 38) that were added to the enclosures mated within an hour. This suggests that receptive females were quickly detected by males. Furthermore, males were quick to mate with these receptive females which are usually rare in a natural population (see OSR above).

A significant correlation was found between the size of males and females in pairs (with non-ovigerous, receptive females) in the field (linear regression $F_{1,17} = 38.41$, $R^2 = 0.693$, $P < 0.001$, n = 19), but not for pairs that mated in the laboratory in the long-term trials ($F_{1,56} = 0.904$, $R^2 = 0.016$, $P = 0.346$, n = 58)(Fig. 5.8). This suggests, that pairs are formed more selectively in the field compared to the laboratory, where all males in the tanks (except three small males) mated with the female that was available at the time irrespective of female size.

***Cyclograpsus lavauxi* and *Helice crassa*:**

Effect of the internal parasite *Portunion* sp. on mating behaviour and reproduction

Over half of the males (23 out of 39, 59.0%) of *C. lavauxi* and 6 out of 27 (22.2%) of male *H. crassa* used in the long-term mating trials without substrate were infested with the parasite *Portunion* sp. No differences were observed in the mating behaviour of parasitised and unparasitised males. In addition, the number of matings did not differ significantly between parasitised and unparasitised males in the long-term trials (two-way ANOVA with parasitism and size as variables: *C. lavauxi*: $R^2 = 0.568$, $F_{1,35} = 0.591$, $P = 0.447$; *H. crassa* (in trials without substrate): $R^2 = 0.638$, $F_{1,23} = 0.632$, $P = 0.438$, male size and parasitism as categorical variables). Furthermore, parasitised males did not show any obvious morphological alterations. However, whether parasitised males transfer the same amount of viable sperm was not assessed.

Parasitised females were castrated, never developed mobile gonopore opercula, and were therefore morphologically not able to mate. The prevalence of *Portunion* sp. varied in female *C. lavauxi* between 13.3% and 40.8% and in *H. crassa* between 5.4% and 13.1% over a one year sampling period (see Chapter 6). Therefore, as these parasitised females do not become receptive, parasitism increased the already highly male-biased OSR even more. This is likely to increase the intensity of male-male competition for the few receptive females that become available at any one time.

Female gonopore morphology

The female gonopore morphology of 13 grapsid species was examined in relation to the duration of sexual receptivity, i.e. whether mating is restricted to certain times by some morphological structures or changes or whether mating is always morphologically possible. Most of the species investigated had fully developed gonopore opercula that were usually immobile and have been observed to become mobile during the mating season (e.g., *Cyclograpsus lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus* (Chapter 4), *H. sexdentatus* (Chapter 2)), or were immobile at the time of examination and are assumed to develop mobile opercula during the mating season (e.g., *Cyclograpsus insularum*, *Eriocheir japonica*, *Geosesarma peraccae*, *Leptograpsus variegatus*, *Plagusia chabrus*, *Planes minutus*) (Table 5.1). Therefore, for these species, mating is morphologically restricted to certain times when immobile gonopore opercula become temporarily mobile. Other species had very small, reduced gonopore opercula which were mobile and appear to allow mating at any time (e.g., *Metopograpsus frontalis*, *M. latifrons*, *M. quadridentatus*) (Table 5.1). In addition, information on the gonopore morphology of other grapsid species was collected from the literature to expand this analysis (Table 5.1). Three

genera (*Cyclograpsus*, *Pachygrapsus*, and *Plagusia*) were found to contain species in both groups (i.e. where mating is morphologically restricted to certain times or always possible). For example, females of all *Cyclograpsus* species have well-developed gonopore opercula, however, in some species the gonopore opercula are usually immobile and become temporarily mobile during the mating season (e.g., *C. insularum* and *C. lavauxi*), whereas in another species these gonopore opercula are apparently always mobile (*C. integer*). This demonstrates, that there is a high variability within the Grapsidae concerning female gonopore structures and the duration of female receptivity, which will have an impact on the mating behaviour and strategy each species can employ.

5.4 Discussion

Cyclograpsus lavauxi: Mating season, mating behaviour, female receptivity and sperm storage

Cyclograpsus lavauxi was found to have one highly synchronised mating season per year, which is a pattern similar to that found in *Hemigrapsus sexdentatus* (Chapter 2). In addition, receptive females were very rare during the reproductive period in the field in both species (e.g., less than 7.2 % receptive females were collected at any one time). As there are no indications that females of these two species leave the sampled intertidal area (see Chapter 2; Nye 1977; McLay 1988), it seems that females are receptive for a very short time in the field, probably less than 24 hours. If females were receptive for longer in the field, i.e. a few days as observed in the laboratory, then more receptive females should have been found during the mating season in the field. Why females are receptive for longer in the laboratory compared to the field needs to be investigated. In either case, such a short receptive period of the females causes a highly male-biased operational sex ratio, which increases male-male competition and the potential for sexual selection. Therefore, although the mating season is highly synchronised, the very short receptive period of females during this time maintains a selective pressure on factors driving sexual selection.

In the laboratory, female *C. lavauxi* that were housed temporarily with males and did not mate were receptive for a significantly shorter period of time than females that mated, although both had a similar range in the duration of receptivity. The opposite was found for two other grapsid species, *Hemigrapsus sexdentatus* (Chapter 3) and *H. crenulatus* (Chapter 4) when females were either isolated from males or males were temporarily or continuously present. An

extended receptive period as is the case of isolated females of *H. sexdentatus* and *H. crenulatus* gives these females more time to find a mate, however, the shortened receptive period of *C. lavauxi* decreases their chance to mate.

Female *C. lavauxi* mated only few times (average of 0.6 matings) during their receptive period in the short-term trials, although they were receptive on average for about a week in the laboratory and had an opportunity to mate every day. The low number of matings of females in the short-term trials might be partially explained by the high interference of males with each other and with newly formed pairs, which might have prevented successful matings, and the limited hiding opportunities in the tanks. In the field, pairs were more likely to hide beneath and between rocks and pebbles and therefore avoid interference by other crabs. The number of female matings (range of 1-3 matings) in the long-term trials is more likely to reflect the real number of matings in the field because all ovigerous females in the field had freshly filled spermatheca (i.e. they all had mated) and their spermatheca weight was in the range of the females that had mated once or twice in the laboratory.

The mean duration of copulations in *C. lavauxi* was about two hours, which was much longer than the average 10 to 15 min reported for the other three NZ grapsid species studied, *H. crassa*, *Hemigrapsus sexdentatus* (Chapter 3) and *H. crenulatus* (Chapter 4). In addition, male *C. lavauxi* held females on average for an hour prior to mating, whereas the other NZ grapsid species mated within minutes. The duration of copulation is also shorter in the grapsid crabs *Sesarma reticulatum* (41.3 min), *S. cinereum* (33.2 min) (Seiple and Salmon 1982). The extended copulation duration of *C. lavauxi* could have a physiological mechanism in the time necessary for sperm transfer. Alternatively, males might use an extended copulation duration to have longer control over the female during her limited time of sexual receptivity because it will reduce the potential for other males gaining access to the female.

***Helice crassa*: Mating season, mating behaviour and female receptivity**

Ovigerous female *H. crassa* were found during the spring and summer months (August to February), which were similar to reports from other sites in New Zealand (Nye 1977; Wear 1970; Jones 1980). During this time, female *H. crassa* typically produce two broods (Nye 1977). The mating season in the field is spread out over several months with few receptive females found at any one time, causing a highly male-biased OSR.

In the laboratory, female *H. crassa* were receptive for up to two weeks prior to oviposition and mated many times during this time, causing high male-male competition and sperm competition within the female spermatheca. Male *H. crassa* fought frequently over females,

which resulted in larger males being more successful in the number of matings, gaining more access to, and defending females better than smaller males. The higher mating success of large males compared to medium and small males has been observed for all four grapsid species from New Zealand, which have been recently studied in long-term trials under similar laboratory conditions, i.e. *Hemigrapsus sexdentatus* (Chapter 3), *H. crenulatus* (Chapter 4), *Cyclograpsus lavauxi*, and *Helice crassa* (Fig. 5.9).

The mating frequency of female *H. crassa* decreased during the receptive period in the laboratory. This could be due to the female either becoming less interested in mating because the spermatheca is relatively full, or becoming less attractive to males, i.e. because the female could be releasing less pheromone. Alternatively, male sexual activity could be decreasing due to a lack of interest in mating repeatedly with the same female or depleted sperm storage due to the high number of copulations (up to 37 times) during the long-term trials.

Matings of *H. crassa* were observed on the substrate surface (surface mating, SM) as well as underground (underground mating, UM) in the laboratory and field. Previously, only surface mating had been observed for *H. crassa* (Beer 1959; Nye 1977) and to my knowledge no other grapsid crab has been observed to mate underground. However, underground matings probably do occur in some of the other burrowing grapsid species, such as *Sesarma reticulatum* (see Seiple & Salmon 1982). In the laboratory, matings were significantly more common on the surface for *H. crassa*, but appeared to be more common underground in the field. However, as only a limited number of matings were observed in the field (n = 6) and it cannot be excluded that a few UM occurred during the observation time, the exact relationship of SM *vs.* UM in the field needs to be established yet. In the laboratory, UMs were not interrupted by other males. Therefore, UM increased a male's chance to successfully mate with a female. In addition, the burrows are likely to provide protection against predators during mating in the field. However, in the laboratory, pairs always separated after mating, even after mating in burrows, which indicates that a particular burrow is not used for mating followed by oviposition. Furthermore, burrows of *H. crassa* appear to be relatively temporary structures in the field because more than 75% of the crabs occupied a burrow for only one day and most of the burrows disappeared after one day (Sivaguru 2000). No crab stayed in the same burrow for more than a week (Sivaguru 2000). This also supports the idea that although burrows are used as sites for mating, they are less likely to be used by males as long-term sites for 'securing' receptive females until oviposition and to ensure paternity by male burrow holders, which as has been suggested for some ocypodid crabs (Koga et al. 1993). In addition, because of the short longevity of the burrows of *H. crassa* (Sivaguru 2000), the same burrow cannot be permanently used as a site for

incubation, as the eggs take about 35 days to develop before the larvae hatch (Nye 1977). Similar to *H. crassa*, some ocypodid crabs mate on the surface as well as underground, e.g., *Uca beebei* (Christy 1987), *Uca lactea* (Murai et al. 1987), and *Scopimera globosa* (Koga 1998), and females often oviposit in their own burrows.

Cyclograpsus lavauxi and *Helice crassa*:

Effect of the internal parasite *Portunion* sp. on mating behaviour and reproduction

Reproduction of female *Cyclograpsus lavauxi* and *Helice crassa* was markedly reduced by the parasite *Portunion* sp., because all parasitised females were castrated. Furthermore, the parasite increases the already male-biased OSR as it eliminates receptive females. Therefore, even fewer females will become receptive and available for mating at any given time during the mating season. However, the parasite had no obvious effect on mating behaviour of parasitised males and male-male competition was not affected by the parasite. This suggests that females did not distinguish between unparasitised and parasitised males. *Portunion* sp. has been reported to have similar effects on the mating behaviour and reproduction on the grapsid crab *Hemigrapsus crenulatus*, which is another host for *Portunion* sp. in New Zealand (see Chapter 4).

Comparison of reproductive strategies within the family Grapsidae s.l.

Mating behaviour of Grapsidae

The mating behaviour of Grapsidae is often restricted to a very short encounter between a male and a female for copulation. Grapsid crabs do not display elaborate courtship behaviour. Typically, a male quickly approaches a female and grasps her without any previous courting, e.g., *Hemigrapsus* spp. (Table 5.2). For some species, a brief courtship display has been observed, such as rhythmical lifting of the chelae by the male (e.g., *Goniopsis cruentata, Grapsus grapsus*, and *Sesarma eumolpe*), rhythmical pushing against the males chelae shield posture by the female (*Eriocheir sinensis*), a dance (e.g., *Eriocheir sinensis, Grapsus grapsus*, and *Pachygrapsus crassipes*), straddling and tapping of females by males (e.g., *Gaetice depressus, Sesarma reticulatum, Sesarma cinereum*,), or the attraction of females with the production of drumming sounds with the walking legs by the male (e.g., *Sesarma curacaoense* and *S. rectum*) (see Table 5.2 for references). Courtship displays have been observed mainly above the water level except for *Eriocheir sinensis* (Schöne 1968). This might be partially explained by easier access for observations and a bias in the number of studies for semi-terrestrial species compared to sub-tidal and oceanic species, which are often more difficult to

investigate (e.g., *Planes* spp. which live on floatsam in the open ocean). In addition, it has not been investigated for any grapsid crabs, whether pheromones are involved in attracting mates. As grapsid females typically do not display their receptivity with a special reproductive behaviour, it is likely that females release chemical cues to advertise their receptivity as has been suggested and shown for other crustaceans (see review Dunham 1978; Christofferson 1978, Imafuku 1986; Bamber & Naylor 1997).

Prolonged post-copulatory guarding by the male has so far only been observed for two species of Grapsidae, *Hemigrapsus crenulatus* and *H. sexdentatus* (Table 5.2). Post-copulatory guarding has been argued to be an important behaviour in ensuring paternity and reducing sperm competition within the female spermathecae (Jivoff 1997b). It is surprising that post-copulatory guarding has not been observed for any other grapsid species so far. However, this might be partly due to the lack of studies. Therefore, post-copulatory guarding might also be found in other grapsid species in the future, e.g., in other *Hemigrapsus* species or in burrowing *Sesarma* or *Armases* species. Males of several burrowing ocypodid species have been reported to plug their burrows after mating or stay with the female until oviposition (e.g., Christy 1983; Koga et al. 1999; Koga 1998) presumably to ensure paternity. As grapsid crabs occupy a diverse range of habitats, a high diversity of sites for mating occurs, e.g., on the surface, in burrows, or under rocks (Table 5.2). Consequently, a variety of mating strategies would be expected depending on the habitat the species occupy, to ensure mating success during inter-sexual competition and to avoid, at the same time, other risks such as predation during mating (see below).

The time of mating within the moulting and reproductive cycle is such that grapsids mate typically during the intermoult, e.g., *Cyclograpsus punctatus* (Brockhuysen 1941), *Metopograpsus messor* (Anilkumar et al. 1999), and *Sesarma ricordi* (Warner 1967) (also all species in Table 5.2 except *Pachygrapsus crassipes*). Furthermore, the time of mating is often directly linked to oviposition and mating occurs in the few days prior to oviposition, e.g., *Cyclograpsus lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus* (Chapter 4), and *H. sexdentatus* (Chapter 3). In addition, some females also mate after oviposition (e.g., *Helice crassa*). The only known exception to this general pattern is *Pachygrapsus crassipes*, which has been observed to mate within hours after the female moulted and on one occasion an 'attempted' copulation while the female was carrying eggs (Hiatt 1948). All other species, which have been tested for sexual receptivity after moulting, were found to be unreceptive soon after moulting, e.g., *Sesarma reticulatum* (Zimmerman & Felder 1991), *Gaetice depressus* (Fukui 1993), and *Hemigrapsus crenulatus* (Chapter 4).

The duration of receptivity in female grapsids is unknown for most species, although two major groups can be distinguished: females for which sexual receptivity is restricted morphologically to certain times (often only few days) and females which are morphologically always able to mate (Table 5.2 & 3). Females of the latter group, however, might not necessarily always be receptive or attractive to males.

Mating strategies of Grapsidae

The ecological and behavioural potential to monopolise mates is a major criterion for the mating strategy of a species (Emlen & Oring 1977). In addition, the combination of male and female strategies or choices will determine the overall reproductive strategy of a species. Christy (1987) grouped mating associations of brachyuran crabs according to the mode of male competition for females. He divided male competition into three general categories with several subdivisions: 1. female centered competition (search & defend, patrol & defend, capture & defend, attract & defend), 2. resource centered competition (breeding site defence, refuge defence), and 3. encounter rate competition, during which males try to maximise the probability of meeting a female but neither defend females nor resources (neighbourhoods, search/interception). Christy (1987) provided grapsid examples for the last two categories, but not for the first one, as at the time there were fewer grapsid species for which comprehensive information was available. The present study enables a more thorough examination of the relationship between the use of certain mating strategies and other behavioural and habitat variables (Tables 5.2 and 5.3). This extended analysis shows male grapsid crabs employ a variety of strategies, and present examples in all three major categories, i.e., female centered, resource centered, and encounter rate competition. Despite these different male strategies, many female grapsids have been observed to mate multiple times and often with different males (Table 5.3, Chapters 3 and 4). Therefore, none of the male strategies, which are all trying to ensure the male's success in fertilisation of the ova of the female, can completely prevent the female from mating with other males. Sperm competition within the female spermatheca therefore is a common feature within the Grapsidae.

The time and duration of female receptivity is expected to have an impact on a male's strategy, because it will influence the time a male might invest in staying with a receptive female by defending or guarding her, or in searching for other receptive females so as to achieve maximum reproductive success (Parker 1974). Furthermore, a male strategy will also depend largely on how sperm is used (i.e. first or last male sperm precedence). However, as for most grapsid species no detailed information is available so far on the exact duration of female

receptivity and sperm precedence. Only limited preliminary conclusions can be drawn at this time when comparing male strategies with female receptivity.

Initially, I expected that a short receptive period of the females (i.e. a few days) would generally elicit a male strategy that would ensure a high probability of paternity such as guarding or defending of receptive females. However, this was not the case, as males of some species followed a search and intercept strategy (e.g., *Cyclograpsus lavauxi*, *Helice crassa*), whereas others guarded and defended females with a short receptive period (e.g., *Hemigrapsus crenulatus*, *H. sexdentatus*) (Table 5.3). Similarly, the occurrence of multiple matings by the females did not appear to influence the male's mating strategy. For example, although the females of many species mated multiple times (Table 5.3), only some species (e.g., *Hemigrapsus sexdentatus*, *H. crenulatus*) employed a male strategy such as guarding that would reduce the probability of the female mating with other males. However, a male's reproductive success in this context will also depend on the way the sperm is stored within the female spermathecae. Most grapsid crabs appear to have ventral-type spermathecae, a type that has been shown to favour the last male's sperm during fertilisation (Diesel 1991; Koga et al. 1993; Urbani 1998). Therefore, a guarding strategy would be expected that will increase a male's fertilisation probability. However, once the exact pattern of paternity for the different grapsid species is established, better predictions can be made.

The habitat is another criterion that is expected to influence a male's strategy to maximise his reproductive success. There are some examples that seem to link species that inhabit a 'stable' or relatively permanent habitat (e.g., crevices, long lasting burrows) with a resource centered competition for females such as males defending crevices (*Pachygrapsus transversus*; Abele et al. 1986) or long lasting burrows (*Sesarma reticulatum*; Seiple & Salmon 1982; Zimmerman & Felder 1991) as sites for mating.

Hemigrapsus sexdentatus and *C. lavauxi* occur both in a rocky habitat (McLay 1988), but at different intertidal zones and are therefore differentially exposed to wave action and air. *Hemigrapsus sexdentatus* lives in the mid-intertidal zone, where it typically hides under stones and boulders during low tide, often in small puddles (McLay 1988). This habitat appears to give *H. sexdentatus* enough protection from desiccation, wave action, and predators to allow males to search for and defend receptive females for a prolonged period of time (female centered competition).

In contrast, *Cyclograpsus lavauxi* lives in the high-intertidal zone among rocks and boulders and is frequently exposed to strong wave action during high tide and to air for several hours during low tide (McLay 1988). *Cyclograpsus lavauxi* backs up diagonally against stones

and holds onto them with his last pair of legs, a behaviour that is thought to stabilise the crab against the effect of the waves and to aid in mobility (McLay 1988). In this habitat, male *C. lavauxi* perhaps cannot easily stabilise themselves and guard receptive females at the same time for a prolonged period of time as they will get washed away by the water during high tide. In addition, females also carry the risk of getting washed away and therefore are likely to resist prolonged guarding attempts by males. Furthermore, the probability of desiccation (during low tide) and predation will be smaller for a single crab that is concealed and protected closely under a rock than a 'bulky' pair trying to fit underneath a stone. Therefore, male *C. lavauxi* follow a mating mode in which they search for and intercept a receptive female only for the duration of mating and then move on until they encounter another receptive female (encounter rate competition: pure search and interception).

The high predation rates on the open mud flats for *Helice crassa* coupled with the short lifetime of burrows (about one day; Sivaguru 2000), which are not used for egg incubation by the females, are likely to be important factors in the evolution of the male mating mode, in which males neither directly compete for females nor for resources. Instead, male *Helice crassa* searched for receptive females in their immediate neighbourhood and mated with them briefly on the substrate or in the burrow after which the female left (encounter rate competition: neighbourhoods of dominance).

H. crenulatus lives in the mid- to low-intertidal zone in sheltered habitats, where it either hides under stones or buries in soft substrates such as mud and clay (McLay 1988). Similar to *H. sexdentatus* this habitat appear to give the crabs enough protection from desiccation, wave action, and predators to allow males to search for and defend receptive females for a prolonged period of time (female centered competition).

Therefore, the different habitats and associated behaviour of finding physical protection from wave action and reducing the risk of desiccation and predation appear to play an important factor in shaping the different mating system of *Hemigrapsus sexdentatus*, *H. crenulatus*, *C. lavauxi*, and *Helice crassa*.

The duration of the reproductive season and the duration of female receptivity of *Hemigrapsus sexdentatus* and *C. lavauxi* are very similar: both have a highly synchronous mating season once per year and females are receptive for only a few days. However, the mating strategies of males of these species are different in that male *H. sexdentatus* guard females and therefore increase their chance of reproductive success, whereas this behaviour does not occur in male *C. lavauxi*. Again, habitat differences are likely to influence the male's strategy. In

addition, a high degree of aggression was observed for *C. lavauxi* in the laboratory, which might cause the female to quickly leave after mating and thereby avoid injury.

Similarly, differences in the mating systems of other species (some of which even belong to the same genus) may be explained by differences in their ecology such as distribution, density, predation, feeding, clutch size and breeding requirements. For example, differences in the mating system of the ocypodid crabs *Uca pugilator* (resource-defence mating system) and *U. vocans* (resource-free mating system) was argued to be caused by the ecology of these species (Christy & Salmon 1984), which was similarly suggested for the grapsid crabs *Sesarma reticulatum* and *S. cenereum*, respectively (Seiple & Salmon 1982).

In summary, grapsid crabs show a high diversity of mating strategies, in which males clearly play the more active part concerning the approach and guarding of females as well as fighting with other males over females. As female duration of receptivity is often restricted to a limited time, a highly male-biased OSR increases male-male competition during which larger males are often more successful. Sperm competition within the female spermatheca, due to multiple mating by the female, was common for many grapsid species. However, so far it appears, that only a few species have developed a behaviour, such as prolonged post-copulatory guarding, or other mechanism that reduces the risk of sperm competition.

Table 5.1 Female gonopore morphology of Grapsidae in relation to the duration of sexual receptivity (time available for mating).

	Species	Reference
Mating morphologically restricted to certain times when immobile gonopore opercula become mobile:		
	Aratus pisoni	Hartnoll 1965
	Armases angustipes *	Hartnoll 1968
	A. cinereum *	Seiple & Salmon 1982; Abele 1992
	A. curacaoense *	Hartnoll 1968
	A. ricordi *	Hartnoll 1965, 1968
	Cyclograpsus insularum	this study
	C. lavauxi	this study
	Eriocheir japonica	this study
	Gaetice depressus	Fukui 1993, this study
	Geograpsus lividus	Hartnoll 1968
	Geosesarma peraccae	this study
	Goniopsis cruentata	Hartnoll 1968
	Grapsus grapsus	Hartnoll 1968
	Helice crassa	this study
	Hemigrapsus crenulatus	this study (Chapter 4)
	H. sexdentatus	this study (Chapters 2 and 3)
	Leptograpsus variegatus	this study
	Pachygrapsus gracilis	Hartnoll 1965
	P. marmoratus	Hartnoll 1968
	Plagusia chabrus	this study
	Planes minutus	Hartnoll 1968, this study
	Sesarma bidentatum	Hartnoll 1968
	S. reticulatum	Seiple & Salmon 1982; Zimmermann & Felder 1991; Abele 1992
	S. verleyi	Hartnoll 1968
Mating always morphologically possible, females have:		
I. no gonopore opercula	*Brachynotus sexdentatus*	Hartnoll 1968
	Percnon gibbesi	Hartnoll 1965, 1968
II. a small / reduced gonopore opercula	*Metopograpsus frontalis*	this study
	M. latifrons	this study
	M. quadridentatus	this study
	Pachygrapsus transversus	Hartnoll 1965, 1968
III. well-developed but always mobile gonopore opercula	*Cyclograpsus integer*	Hartnoll 1965, 1968
	Metopaulias depressus	Hartnoll 1965, 1968
	Plagusia depressa	Hartnoll 1965

* formerly placed in the genus *Sesarma* but now placed in *Armases* by Abele (1992)

Table 5.2. The occurrence of a short courtship display and prolonged post-copulatory guarding in grapsid crabs. Courtship is referred to if it is anything more than a male simply approaching a female, touching the female with his walking legs and before embracing the female for copulation.

Species (sorted by the absence or presence of male courtship)	Short courtship display	Prolonged post-copulatory guarding	Location of observed field matings:	Reference
Cyclograpsus lavauxi	-	-	underneath rocks and boulders	this study
Eriocheir japonica	-	?	?	Morita 1974 as cited in Fukui 1994
Helice crassa	-	-	on surface and in burrows	Nye 1977, this study
Hemigrapsus crenulatus	-	+	?	this study (Chapter 4)
H. nudus	-	-	underneath large stones	Knudsen 1964
H. oregonensis	-	-	below water level	Knudsen 1964; Lindberg 1980
H. sexdentatus	-	+	underneath rocks and boulders	this study (Chapter 3)
Pachygrapsus transversus	-	-	at entrance of female's hole/crevice	Abele et al. 1986
Eriocheir sinensis	+	-	below water level	Schöne 1968
Gaetice depressus	+	-	?	Fukui 1994
Goniopsis cruentata	+	-	above water level	Schöne & Schöne 1963; Schöne 1968
Grapsus grapsus	+	-	above water level	Kramer 1967; Schöne 1968
Pachygrapsus crassipes	+	-	above water level	Bovbjerg 1960; Schöne 1968
Sesarma cinereum	+	-	on surface	Seiple & Salmon 1982
S. curacaoense	+	-	?	von Hagen 1967
S. eumolpe	+	-	?	Verwey 1930, as cited in Hartnoll 1969
S. rectum	+	-	?	von Hagen 1967
S. reticulatum	+	-	on surface; speculated to be in male burrows	Seiple & Salmon 1982; Zimmerman & Felder 1991
Aratus pisoni	?	-	on mangrove roots	Warner 1967

Present, +; not present, -.

Table 5.3. Mating associations of grapsid crabs sorted by the mode of male competition for females. Females of all of these species mate during the intermoult, except *Pachygrapsus crassipes*, which also mates in the hours after moulting.

Mating association[1]	Species	Duration of female receptivity	Occurrence of multiple matings by the female	References
1. Female centered competition				
Search and defence of mobile females	*Hemigrapsus crenulatus*	temporary (few days)	+	this study (Chapter 4)
	Hemigrapsus sexdentatus	temporary (few days)	+	this study (Chapter 3)
2. Resource centered competition				
Defence of refuges	*Pachygrapsus transversus*	permanent	?	Abele et al. 1986
	Sesarma reticulatum	temporary[2] (few days)	+	Seiple & Salmon 1982; Zimmerman & Felder 1991
3. Encounter rate competition				
Neighbourhoods of dominance	*Aratus pisoni*	temporary	?	Hartnoll 1965; Warner 1967, 1970
	Goniopsis cruentata	temporary	?	Hartnoll 1965; Schöne & Schöne 1963; Warner 1967, 1970
	Helice crassa	temporary (few weeks)	+	Beer 1959; Nye 1977; this study
Pure search and interception	*Cyclograpsus lavauxi*	temporary (few days)	+	this study
	Gaetice depressus	temporary (few days)	-[3]	Fukui 1993, 1994
	Grapsus grapsus	temporary	?	Hartnoll 1965; Kramer 1967
	Hemigrapsus nudus	?	?	Knudsen 1964
	Hemigrapsus oregonensis	?	+	Knudsen 1964; Lindberg 1980
	Pachygrapsus crassipes	?	+	Hiatt 1948; Bovbjerg 1960
	Sesarma cinereum	temporary[2]	+	Seiple & Salmon 1982

1, as defined by Christy (1987); 2, in addition, attempted copulations with females with immobile opercula have been observed; 3, not during a two-hour experiment

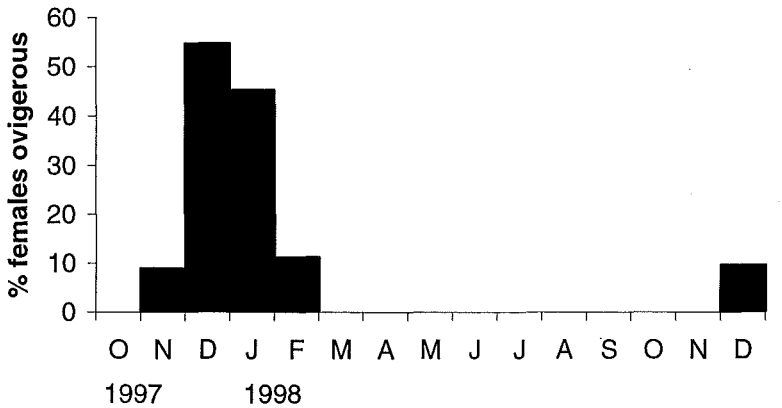


Figure 5.1 Percentage of ovigerous female *Cyclograpsus lavauxi* from October 1997 to December 1998.

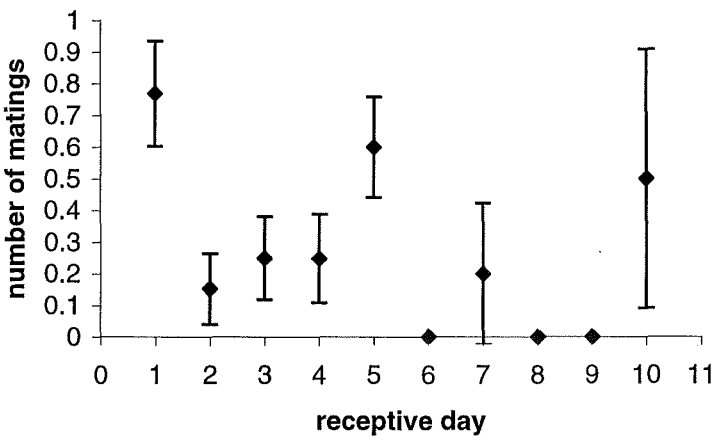


Figure 5.2 Mean number of matings (±S.E.) on each day of the receptive period until oviposition of female *Cyclograpsus lavauxi* during the long-term trials.

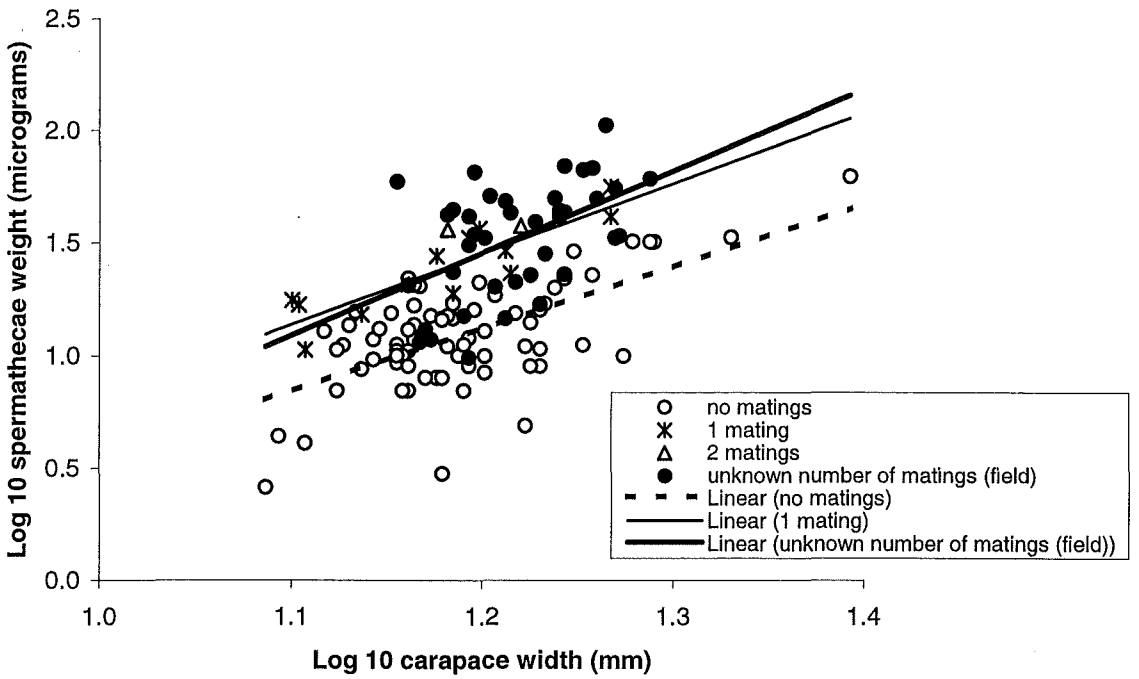


Figure 5.3 Spermatheca weight of ovigerous female *Cyclograpsus lavauxi* from the field and laboratory (linear regression equations: no matings (n = 73), y = 2.7755x – 2.21; 1 mating (n = 13), y = 3.1309x – 2.3064; field (unknown number of matings (n = 39), y = 3.6448x – 2.9189).

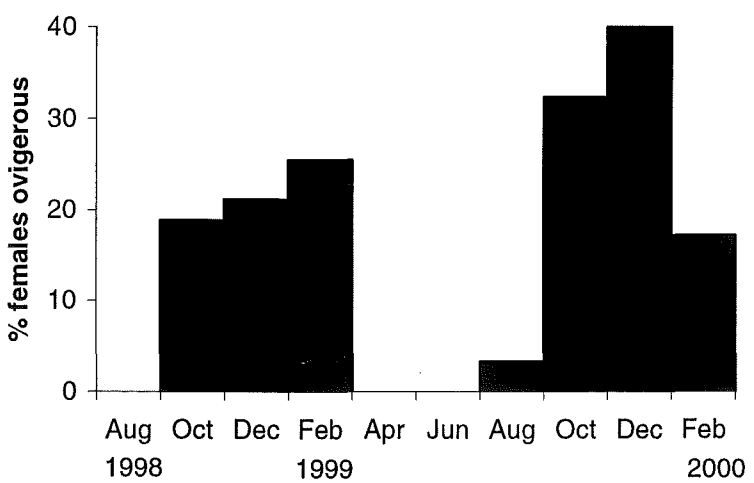


Figure 5.4 Percentage of ovigerous female *Helice crassa* from August 1998 to December 1999.

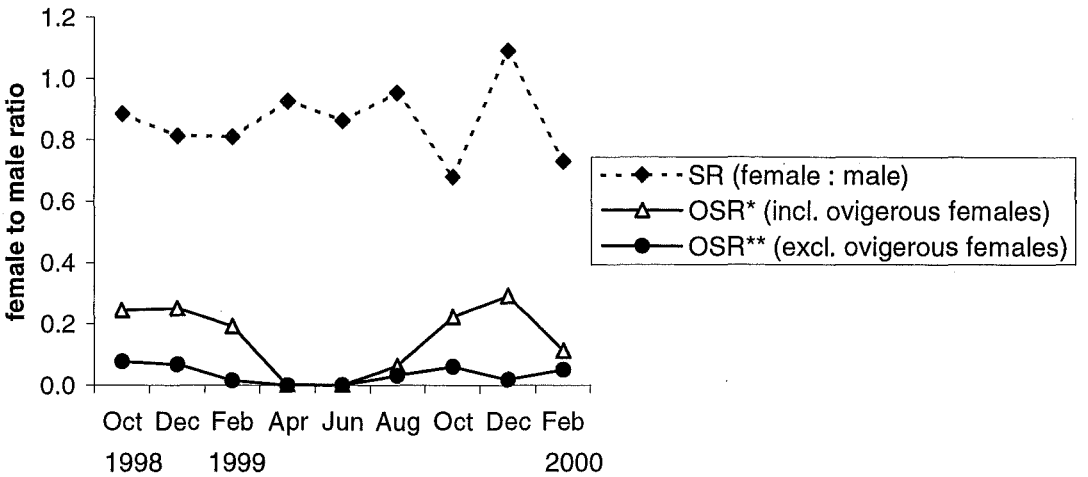


Figure 5.5 Sex ratio (SR) and operational sex ratio (OSR) of mature *Helice crassa* collected from transects from 1998 to 2000. The operational sex ratio (OSR) was divided into two types: OSR* refers to all receptive females (with and without eggs), whereas OSR** refers only to receptive females without eggs.

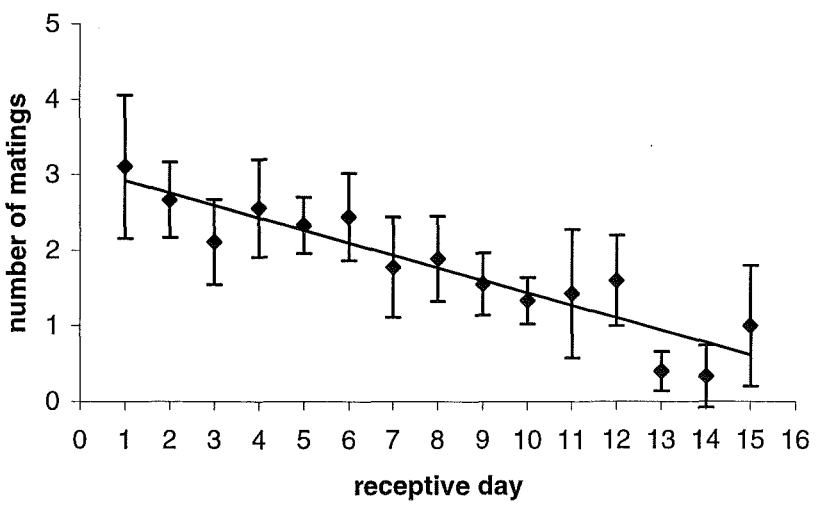


Figure 5.6 Mean number of matings (±S.E.) on each day of the receptive period until oviposition of female *Helice crassa* during the long-term trials (without substrate). Linear regression equation: $y = -0.1602 + 3.0291$, $R^2 = 0.832$.

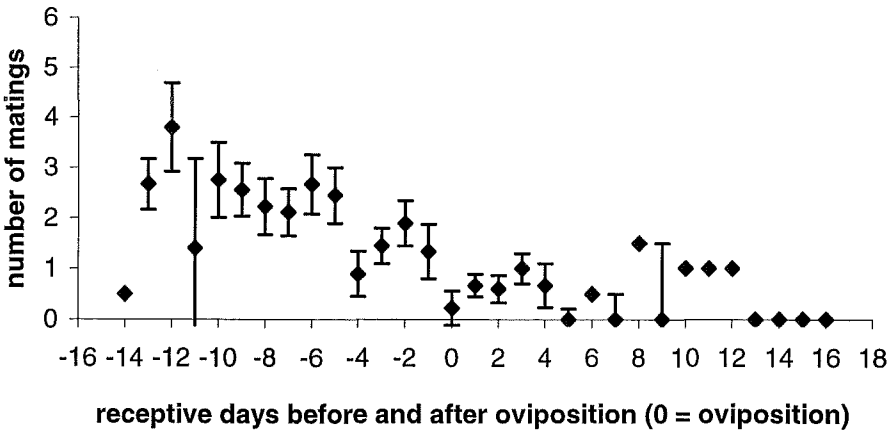


Figure 5.7 Mean number of matings (±S.E.) on each receptive day before and several days after oviposition of female *Helice crassa* during long-term trials (without substrate). Eggs were laid on day zero.

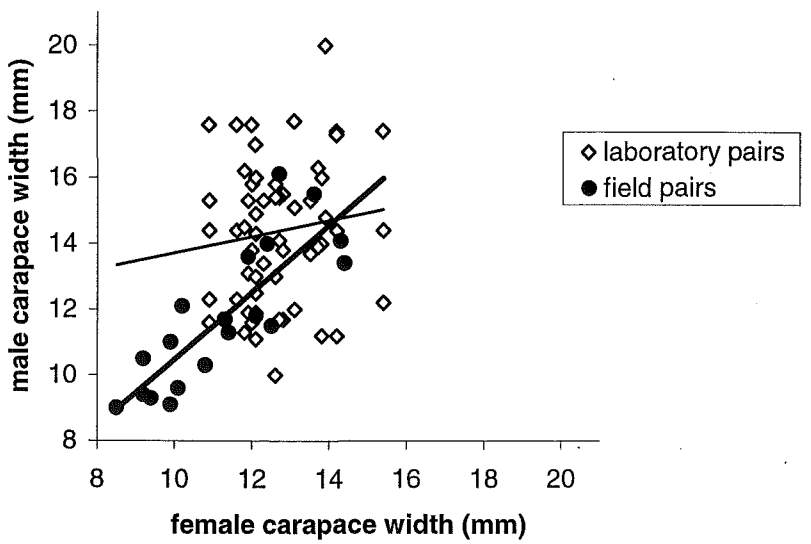


Figure 5.8 Size of male and female pairs of *Helice crassa* observed in the laboratory during long-term trials (n = 58, trials with and without substrate combined) and field (n = 19). Linear regressions: laboratory pairs, y = 0.2463x + 11.254; field pairs, y = 1.021x + 0.2633.

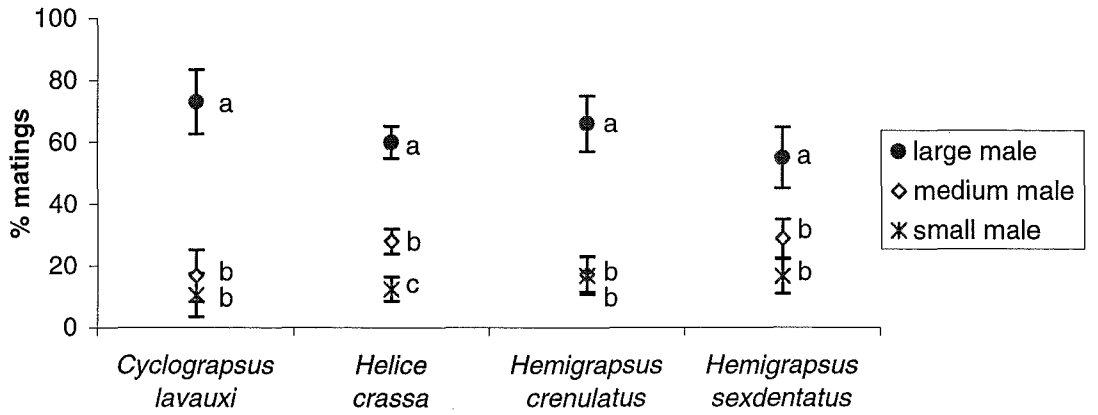


Figure 5.9 Relative mating success of large, medium and small males of *Cyclograpsus lavauxi* (n = 13), *Helice crassa* (n = 9), *Hemigrapsus crenulatus* (n = 11), and *Hemigrapsus sexdentatus* (n = 14) in the presence of one female in long-term trials (without substrate) until oviposition. Operational sex ratio of one female to three males. The letters indicate statistically significant differences within a species.

6 Ecology of the internal parasite *Portunion* sp. (Isopoda: Entoniscidae) and its effect on reproduction in intertidal crabs (Decapoda: Grapsidae) from New Zealand

Abstract - The parasite fauna of four intertidal grapsid crabs from New Zealand was studied between 1998 and 2000. Here, results are presented on the occurrence and impact of an undescribed entoniscid species, *Portunion* sp. (Isopoda: Epicaridea). *Portunion* sp. was found in 34.1% of *Cyclograpsus lavauxi* (n = 1650), 19.0% of *Hemigrapsus crenulatus* (n = 2300), and in 11.6% of *Helice crassa* (n = 825), but was absent in *Hemigrapsus sexdentatus* (n = 636). Parasitised hosts contained mostly one female *Portunion* sp., but occasionally up to seven females were found. One to three dwarf males occured on a mature female *Portunion* sp. Parasite prevalence was generally higher in male hosts than in female hosts and increased significantly with host size. Most developmental stages of female *Portunion* sp. were found throughout the year, indicating that reproduction and infection occurs continuously. *Portunion* sp. differentially affected male and female hosts. Females were castrated, whereas males were not. *Portunion* sp. therefore changes the operational sex ratio in its host species, causing a more male-biased ratio which is likely to affect the intensity of male-male competition.

6.1 Introduction

Entoniscid isopods (Epicaridea) are unusual internal parasites living in the hemocoel of decapod crustaceans, such as true crabs, hermit crabs, and one species of alpheid shrimp. Larval and male entoniscids possess the general isopod characters of a dorsoventrally compressed body, segmentation, and pereiopods, whereas females are strongly modified and hardly recognisable as isopods. For example, the unsegmented thoracic region of adult female entoniscids is surrounded by large oostegites bounding the brood pouch and forming extensive lobes. Female entoniscids are surrounded by a host response sheet (Kuris et al. 1980). Although many of the known entoniscid isopods have been described many years ago (e.g., Kossman 1881, Giard and Bonnier 1886; Drach 1941; Shiino 1942; Muscatine 1956), there are few studies available on their life cycles and interactions with hosts (e.g., Atkins 1933; Veillet 1945; Kuris et al. 1980). Consequently, the life cycle of most Entoniscidae is only partly known or completely unknown. It is assumed that most entoniscids have a copepod intermediate host like other Epicaridea

(Overstreet 1983; O'Brian & van Wyk 1985). Veillet (1945), who studied the entoniscid *Portunion maenadis* in the swimming crab *Carcinus maenas*, was able to show that the epicaridea larva attaches itself to the copepod *Acartia* which is the intermediate host for this species. After metamorphosis into a microniscus larva on the copepod, the free living cryptoniscan larva then infects and penetrates the final host directly, presumably through the gills (Veillet 1945). After infestation, a series of transformations of the female parasite follows, including an asticot, juvenile and adult stage, resulting in the typical female entinoscid form. Dwarf males live in and around the female's brood pouch (marsupium). Eventually, epicaridan larvae are released into the water through a pore that connects the female parasite with the thin cuticle of the host gill chamber (Caullery 1952). Parasitic castration has been reported for many entoniscids (e.g., *Portunion conformis* in *Hemigrapsus oregonensis*, Kuris et al. 1980), particularly with respect to the female host.

Aquatic parasites and diseases of New Zealand marine animals have received only limited attention in the past, although several of the commercially important fish and shellfish species have been investigated with regard to parasites and diseases (Hine & Jones 1994). Crustacea in particular are poorly studied in this respect. So far there has not been any published report of entoniscids in New Zealand.

A parasitological survey was carried out on four New Zealand intertidal crabs of the family Grapsidae: the smooth shore crab *Cyclograpsus lavauxi*, the hairy handed crab *Hemigrapsus crenulatus*, the purple rock crab *Hemigrapsus sexdentatus*, and the tunnelling mud crab *Helice crassa*. Here, I report on the occurrence of an undescribed entoniscid isopod in the genus *Portunion*. Currently there are seven known species in this genus. The temporal prevalence of *Portunion* sp. in its hosts and the relationship of parasite prevalence to host gender and size were studied. The effect of *Portunion* sp. on host reproduction was also investigated.

6.2 Materials and Methods

The four intertidal grapsid crab species were collected from four sites on the east coast of the South Island of New Zealand between 1998 and 2000 where one or two of these species were common. A total of 1650 *Cyclograpsus lavauxi* and 1970 *Hemigrapsus crenulatus* from Governors Bay in Lyttelton Harbour (43° 38′ S, 172° 39′ E), 825 *Helice crassa* from the Avon-Heathcote Estuary, and 636 *Hemigrapsus sexdentatus* from a beach near Waipara (43°06′ S,

172°53′ E) were dissected. In addition a smaller sample of 21 *Cyclograpsus lavauxi* was dissected from Kaikoura (42° 23′ S, 173° 39′ E) in 1999.

Collection methods varied depending on the habitat of each species. *Cyclograpsus lavauxi* were collected monthly by hand for a year during low tide from underneath rocks along the intertidal zone in Governors Bay (Table 6.1). *Hemigrapsus crenulatus* were collected monthly for a year during high tide using baited net traps (Table 6.1). *Helice crassa* larger than 76 mm CW were collected by hand every two months over a 10 month period during low tide, using quadrats (0.3 m x 0.3 m) every two metres along a 40 m transect (perpendicular to the shoreline) (Table 6.1). *Hemigrapsus sexdentatus* were collected by hand during low tide from underneath rocks in the intertidal zone usually in the first half of three years (Table 6.1). All *C. lavauxi* and *Helice crassa* collected were subsequently dissected. Some of the large collection of *Hemigrapsus crenulatus* (see also below) and *Hemigrapsus sexdentatus* were not dissected because of other ongoing experiments. However, overall a relatively large number of crabs was examined because this study was part of a larger parasitological survey which explored also other parasites of which some were rare and required a larger sample size to be detected.

Crabs were taken to the laboratory where they were measured (CW, using a Mitutoyo digital callipers to the nearest 0.1 mm) and sexed by assessing the relative abdomen width (females have a wider/broader abdomen than males). The reproductive stage of the female was determined by assessing whether females were ovigerous or not. Crabs were usually held under a 12 h light-dark cycle in tanks with circulating seawater of 12 - 15°C in the laboratory. Males and females were kept in separate group tanks and fed opened blue mussels (*Mytilus edulis*) two or three times a week until dissection. The majority of crabs was dissected within two weeks of the date of collection, but some were dissected up to five months after collection because they had been used for behavioural experiments (crab mating behaviour) for which they had temporarily been transferred to other tanks and held under similar conditions (see below for additional information on *Hemigrapsus crenulatus*). Therefore, some of the crabs that were dissected later contained parasites which had additional time to develop further. However, all stages including the early asticot stage (see below) were found in the crabs several weeks after collection. As no data are available on the duration of the developmental stages (see below) of *Portunion* sp., these were recorded as found when dissected. As *Portunion* sp. has most likely an intermediate copepod hosts which does not occur in the laboratory system, it is assumed that crabs could not become infected while in captivity.

Before dissection crabs were killed by placing them in a freezer at –15°C for about 1 hour. The number and developmental stage of *Portunion* sp. as well as the host's size (CW) and

gender were recorded. The internal organs of the hosts were examined under a binocular microscope at 160×. Five developmental stages of female *Portunion* sp. were recognised: asticot, juvenile, large juvenile, adult, and ovigerous adult, following the descriptions of developmental stages for *P. conformis* in *Hemigrapsus oregonensis* (Kuris et al. 1980), i.e., Asticot: C-shaped body, gills as rudimentary, simple lamellae; Juvenile: C-shaped or straight body, gills as simple lamellae or crenulated and frilled shape, oostegites as small, simple lamellae or prominent; large juvenile: straight or V-shaped body, frilled gills, anterior lobe of 1st oostegite extends antero-dorsal to cephalogaster or marsupium closed, ovary developing, white to pale yellow; adult: V-shaped body, frilled gills, empty marsupium of adult capacity, ovary prominent yellow, ova distinct); ovigerous adult: V-shaped body, frilled gills, filled marsupium, ovary white to yellow. In addition, the content of the brood pouch (marsupium) of ovigerous females was divided into recently extruded eggs, embryos, and larvae.

Female crabs that were ovigerous or developed mobile gonopore opercula in the laboratory, indicating that they had become sexually receptive, were never found to be parasitised. Hence, the additional 330 ovigerous or sexually receptive *Hemigrapsus crenulatus,* collected between June and October 1998, were not dissected because it was apparent that they could not have been parasitised by *Portunion* sp. Results of parasite prevalence in *H. crenulatus* refer to the total number of collected females (n = 1046).

Parasitological terms are used as in Bush *et al.* (1997). Prevalence and intensity of *Portunion* sp. refer to the number of female *Portunion* found in the hosts. Prevalence was defined as the number of host infected with one or more female parasite divided by the number of host examined and the mean intensity as the total number of female parasites found in a sample divided by the number of hosts infected. The dwarf males, of which typically one to three were found on a mature female, were not counted as extra parasites and were not included in calculations of parasite intensity and other parameters. Therefore, when *Portunion* sp. is mentioned in the text, it refers to the female of *Portunion.* SYSTAT 9 was used for statistical analysis. Percentages were arcsine transformed before statistical analyses were carried out.

6.3 Results

Portunion sp. were found in 34.1% (562 out of 1650) of *Cyclograpsus lavauxi*, 19.0% (438 out of 2300) of *Hemigrapsus crenulatus* from Lyttelton Harbour, and in 11.6% (96 out of 827) of *Helice crassa* from the Avon-Heathcote Estuary. In addition, 57.1% (12 out of 21) of *C. lavauxi*

were parasitised by *Portunion* sp. in the smaller sample collected from Kaikoura (range of carapace width 14 - 24 mm). *Portunion* sp. was not found in *Hemigrapsus sexdentatus* (n = 636). Careful study of the external morphology showed that the same undescribed species of *Portunion* sp. (Fig. 6.1) was found in all three hosts, indicating that this species is not host specific.

The prevalence of *Portunion* sp. varied between 25.5% and 42.5% over the year for *Cyclograpsus lavauxi*, between 9.9% and 32.3% for *Hemigrapsus crenulatus*, and between 4.1% and 16.0% for *Helice crassa* (Fig. 6.2). No distinct seasonal pattern in parasite prevalence was apparent in any of the three host species (Figs. 6.2 and 6.3).

Male crabs had generally higher parasitism rates than female crabs (Fig. 6.3). A significant difference in parasite prevalence between male and female crabs was found for *C. lavauxi* (Paired *t* test: $t_{11} = 4.76$, $P = 0.001$) and *Hemigrapsus crenulatus* (Paired *t* test: $t_{11} = 6.81$, $P < 0.001$), but not for *Helice crassa* (Paired *t* test: $t_5 = 1.42$, $P = 0.216$). Parasite prevalence differed significantly among the three host species for both males (ANOVA: $F_{2, 27} = 24.516$, $P < 0.001$) and females (ANOVA: $F_{2,27} = 24.687$, $P < 0.001$). Male and female *Cyclograpsus lavauxi* had significantly higher parasite prevalence than those of *Hemigrapsus crenulatus* and *Helice crassa* (Table 6.2). Male *Hemigrapsus crenulatus* had significantly higher parasite prevalence than male *Helice crassa* (Table 6.2).

Most parasitised crabs were infected by one female *Portunion* sp. but occasionally two to seven female parasites were found in one crab. Single parasites were found in 87.4% of the parasitised *C. lavauxi*, 94.1% of the parasitised *Hemigrapsus crenulatus*, and in 93.8 % of the parasitised *Helice crassa*. The average number of *Portunion* sp. per infected crab was 1.16 in *C. lavauxi*, 1.06 in *Hemigrapsus crenulatus,* and 1.08 in *Helice crassa*. If multiple infection by female parasites occurred, the parasites were either in the same or different developmental stage, such as two ovigerous females or a juvenile and an ovigerous female. This suggests that multiple infections do not necessarily occur at the same time and that parasitism does not preclude subsequent infections. Alternatively, unequal development rate may occur when several females are 'crowded' in the same host.

A significant positive correlation was found for *Portunion* sp. prevalence and host size for all three host species (Fig. 6.4) (linear regression: *C. lavauxi*: $R^2 = 0.986$, $P < 0.001$, n = 1647; *Hemigrapsus crenulatus*: $R^2 = 0.929$, $P < 0.001$, n = 2299; *Helice crassa*: $R^2 = 0.583$, $P = 0.027$, n = 822; male and female hosts combined; size classes with less than five crabs not included). Larger and therefore older crabs were more likely to be parasitised than younger crabs.

Most of the developmental stages of female *Portunion* sp. were found throughout the year in *C. lavauxi* and *Hemigrapsus crenulatus*. However, the early stages (i.e. asticot and juvenile) of female *Portunion* sp. were not found during spring and summer in *Helice crassa*. The majority of female *Portunion* sp. were adults and ovigerous adults in the three hosts (Fig. 6.5). The brood pouch of the female parasite was filled with all three developmental stages (eggs, embryos, and larvae) throughout the year for *C. lavauxi* and *Hemigrapsus crenulatus*, and in all months except December and April for *Helice crassa* (Fig. 6.6). This indicates that *Portunion* sp. reproduces throughout the year. Occasionally a few epicaridean larvae from a previous brood were found in the brood pouch together with newly deposited eggs, suggesting that *Portunion* sp. is iteroparous, reproducing several times during their lifetime. Furthermore, as early stages of *Portunion* sp. were found during most months of the year, it appears that the hosts become infected throughout the year. Therefore, no seasonal pattern in reproduction was found for *Portunion* sp. Early stages of female *Portunion* sp. were found in all host size classes, indicating that younger and older crabs can become infected.

A few parasitised *C. lavauxi* and *Hemigrapsus crenulatus* were observed moulting successfully, showing that *Portunion* sp. does not inhibit moulting. No gonads were present in most female hosts. However, in a few instances when a female host carried the early asticot parasite stage, and had therefore just recently been infected, small remnants of the gonad could be detected. No pathology was found in parasitised male hosts and the shape and length of the vasa deferentia of males appeared to be similar to unparasitised males and contained presumably viable sperm. Morphological alteration of the secondary sexual characters, e.g. chelae, were not observed. Therefore, *Portunion* sp. differentially affected male and female hosts. Females were castrated, whereas males were not.

6.4 Discussion

The species of *Portunion* was the same in all crabs investigated and appears to be different from the seven currently described species of *Portunion*, i.e., *P. bourdoni* Chaix & Veillet 1981, *P. conformis* Muscatine 1956, *P. flavidus* Shiino 1942, *P. kossmani* Giard & Bonnier 1886, *P. maenadis* Giard & Bonnier 1886, *P. moniezii* (Kossman 1881), and *P. salvatoris* (Kossman 1881). This discovery of *Portunion* sp. is the first published record of the occurrence of a species of the family Entoniscidae in New Zealand. Previously, the closest other records of

entoniscid isopods have been from islands off the east coast of Australia (*Cancrion australiensis* (Shields & Earley 1993) and *Tiarinion texopallium* (Shields & Ward 1998)).

Portunion sp. was very common in three of the four grapsid species investigated in this study and absent in one. The prevalence of *Portunion* sp. in *Cyclograpsus lavauxi* was significantly higher than that of *Hemigrapsus crenulatus*, although these two species were collected from the same site. However, *C. lavauxi* inhabits mostly the high-shore zone whereas *Hemigrapsus crenulatus* tends to live in the mid- to low-shore zone. Therefore, it would be expected that *C. lavauxi* would have a lower parasite prevalence because it is less exposed to the aquatic infective stages of *Portunion* sp. compared to *Hemigrapsus crenulatus*. However, it might be that *C. lavauxi* is only briefly exposed to the infective stages during high tide, but then has less effective means to clean its exoskeleton and gills (possible sites of host penetration of entoniscids) while exposed to air for long periods. *Cyclograpsus lavauxi* conserves branchial water by recirculation while exposed to air, however, the branchial chambers of some crabs can be found air-filled in dry weather (Innes et al. 1986). Therefore, the maxillipedal epipods which are commonly used in brachyuran decapods for gill cleaning (Bauer 1981, 1989) might not be as effective under these conditions. In contrast, *Hemigrapsus crenulatus* while almost constantly exposed to the infective stages, being under water might provide it with better opportunities to clean itself of the infective stages and prevent the parasite from entering. Ritchie and Høeg (1981) were able to demonstrate that gill cleaning by the chelate fifth pereiopod of the anomuran *Petrolisthes cabrilloi* was an effective means of eliminating infective stages of the rhizocephalan parasite *Lernaeodiscus porcellanae*. More detailed studies investigating the susceptibility of the grapsid crabs to *Portunion* sp. such as the effectiveness of host cleaning of crabs submerged in water and exposed to air, would be useful to explain these prevalence differences for *C. lavauxi* and *Hemigrapsus crenulatus* which were collected from the same site.

Helice crassa was collected from mid to high shore from an estuary and had, on average, the lowest prevalence of *Portunion* sp. among the three species that were infected. The lower salinity of the estuary in which this species was collected could be less favourable for an intermediate host and the infective larval stage and could be one reason to explain this difference. However, salinity is one factor among many, such as temperature and host susceptibility, which may cause differences in parasite prevalence in hosts and further studies are necessary to elucidate these differences. The high parasite prevalence in *C. lavauxi* found in the small sample from Kaikoura is most likely explainable by the relative large size of the crabs which had generally higher parasitism probabilities.

A positive relationship was found between parasite prevalence and host size for all three host species. The same trend was found for *Portunion conformis* in *Hemigrapsus oregonensis* (Kuris et al. 1980) and for *P. kossmanni* in *Portumnus variegatus* (Portunidae) (Sansin 1938 as cited in O'Brien & van Wyk 1985). The increase of prevalence with host size is likely to be related to increasing host age and therefore the result of cumulative exposure to the parasite. O'Brien & van Wyk (1985) list some alternative explanations for increased prevalence with host size such as enhanced growth or survivorship of parasitised hosts or biased sampling of large parasitised hosts because of behavioural differences within the host population. However, no comparative data on growth or moulting frequency of infected and uninfected hosts is currently available for the species studied here. The mating behaviour and number of matings of infected male crabs is the same as that of uninfected crabs (see Chapters 3 to 5).

The majority of the parasites found in the crabs were adult females, and typically most of the females were ovigerous. This shows that *Portunion* sp. reproduces throughout the year. Although the sample size is very small (n = 5), all female *Cancrion australiensis* (Entoniscidae) found in the swimming crab *Thalamita sima* were gravid (Shields & Earley 1993). However, as these entoniscids had been collected for only a limited time (3 months), it is not known whether they produce continuously similarly to *Portunion* sp.

Male hosts had significantly higher *Portunion* sp. prevalence than female hosts in two out of the three infected crab species. It is not known why these differences occur, particularly as male and female crabs inhabit the same habitat. In contrast, generally more female than male *Hemigrapsus oregonensis* were found to be parasitised by *Portunion conformis* (32.9% *vs.* 17.7%, respectively, Muscatine 1956; 29.7% *vs.* 18.6%, Kuris et al. 1980).

Male and female hosts were differentially affected by *Portunion* sp. Females were castrated, whereas in males no reproductive or other pathological effect could be observed. Similarly, *P. conformis* castrated their female host *Hemigrapsus oregonensis*, but had less noticeable affect on their male host although some large males exhibited some feminisation of secondary sexual characters (abdominal width and chela length)(Kuris 1971 as cited in Kuris et al. 1980).

Host castration can be achieved by the parasite directly feeding on the gonads of the host or indirectly by diverting energy away from gonad development, or by influencing the host hormonal balance (Baudoin 1975; Coustau et al 1991; Schallig et al 1991). In addition, some parasitic castrators are thought to prolong host life and cause gigantism of their hosts (Poulin 1988). This has been argued to be beneficial to some parasitic castrators as they then reproduce

for longer and reproduce more offspring, because of the host size-dependent parasite fecundity (Kuris 1974).

It is not known why the genders were differentially affected by *Portunion* sp., as one would expect that the benefits of castration that *Portunion* gains would apply to male and female hosts. However, as the energy necessary for gonad development is most likely higher in female than in male hosts, the parasite would benefit more by castrating the females. Changes in the host, whether they are parasite induced or side effects of infection, and the reasons for them are often not fully understood (Poulin 1988; Moore & Gotelli 1990). More studies are necessary to explain the different effects on female and male hosts by *Portunion*.

A parasitic castrator can influence the sex ratio of their host population by affecting the number of females or males or both differentially. In castrating only female hosts, *Portunion* sp. causes the general sex ratio (Table 6.3) and consequently the operational sex ratio (i.e., ratio of fertilisable females to sexually active males; Emlen & Oring 1977) of their hosts to be more male-biased. However, the mating behaviour of parasitised male hosts was not affected and parasitised males were equally successful when competing for females compared to unparasitised males (see Chapters 3 to 5). Despite the parasite's apparent negative impact on female crab reproductive rates, all three crab species are relatively common at the study site. However, it is likely that the *Portunion* sp. plays an important role in host population regulation as has been suggested for other parasitic castrators (Kuris 1974).

Table 6.1 Number of male and female *Cyclograpsus lavauxi, Helice crassa, Hemigrapsus crenulatus,* and *Hemigrapsus sexdentatus* dissected between 1998 and 2000.

Date of collection		Total numbers examined (males/females)			
		Cyclograpsus lavauxi	*Helice crassa*	*Hemigrapsus crenulatus*	*Hemigrapsus sexdentatus*
1998	Jan	296 (150 / 146)			144 (44 / 100)
	Feb	55 (28 / 27)			107 (50 / 57)
	Mar	113 (63 / 50)			81 (9 / 72)
	Apr	106 (64 / 42)			78 (10 / 68)
	May	90 (51 / 39)			
	Jun	124 (62 / 62)		252 (190 / 62)	
	Jul	111 (62 / 49)		150 (114 / 36)	
	Aug	148 (79 / 69)		120 (85 / 35)	
	Sept	146 (84 / 62)		113 (73 / 40)	
	Oct	116 (55 / 61)	147 (69 / 78)	132 (117 / 15)	
	Nov	100 (55 / 45)		155 (107 / 48)	
	Dec	245 (151 / 94)	114 (52 / 62)	262 (140 / 122)	15 (0 / 15)
1999	Jan			114 (69 / 45)	
	Feb		123 (55 / 68)	176 (99 / 77)	5 (0 / 5)
	Mar			198 (98 / 100)	18 (0 / 18)
	Apr		156 (75 / 81)	184 (100 / 84)	
	May			114 (62 / 52)	
	Jun		162 (75 / 87)		
	Jul				
	Aug		125 (61 / 64)		
2000	Mar				32 (32 / 0)
	Apr				63 (44 / 19)
	May				93 (0 / 93)
	Dec				
	Total	1,650 (904 / 746)	827 (387 / 440)	1,970 (1,254 / 716)	636 (189 / 447)

Table 6.2 Comparison of *Portunion* sp. prevalence among males and females of *Cyclograpsus* lavauxi, *Hemigrapsus crenulatus* and *Helice crassa*. Statistical results of protected Tukey post-hoc test (males / females).

	Hemigrapsus crenulatus	*Helice crassa*
Cyclograpsus lavauxi	$P = 0.002$ / $P < 0.001$	$P < 0.001$ / $P < 0.001$
Hemigrapsus crenulatus		$P = 0.003$ / $P = 0.750$

Table 6.3 Comparison of apparent and actual sex ratio corrected for *Portunion*-induced castration of females of three grapsid crabs.

Host species	Female to male ratio	
	apparent	actual
Cyclograpsus lavauxi	0.83	0.61
Hemigrapsus crenulatus	0.83	0.74
Helice crassa	0.88	0.79

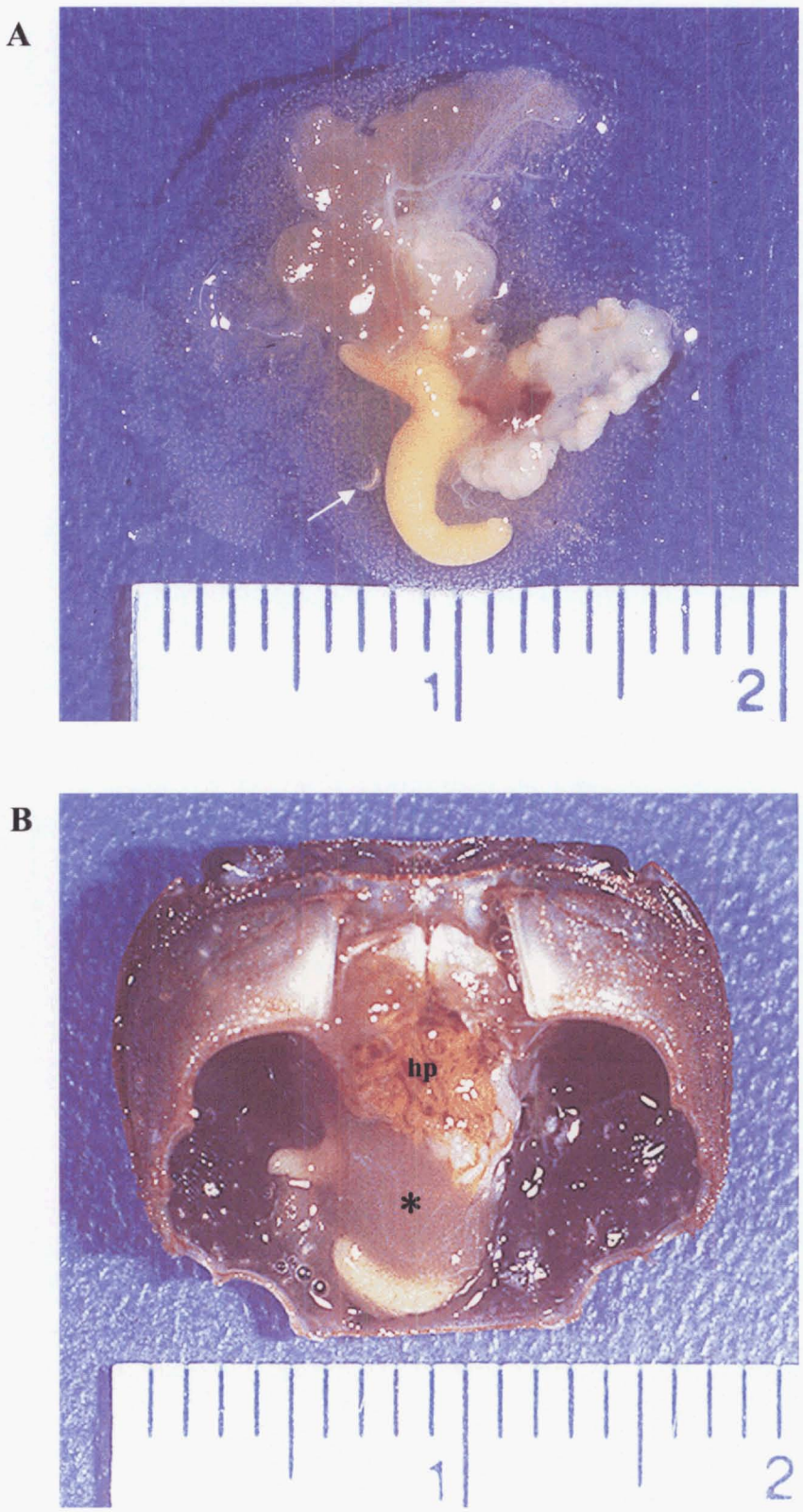


Figure 6.1 *Portunion* sp. (Isopoda: Entoniscidae). A. Adult female *Portunion* sp. with dwarf male (arrow) among embryos (brood pouch ruptured); B. Typical position of adult female *Portunion* sp.(*) in its host, e.g., *Cyclograpsus lavauxi* (host hepatopancreas -hp- partially removed). Scale given by cm rule (with mm subdivisions).

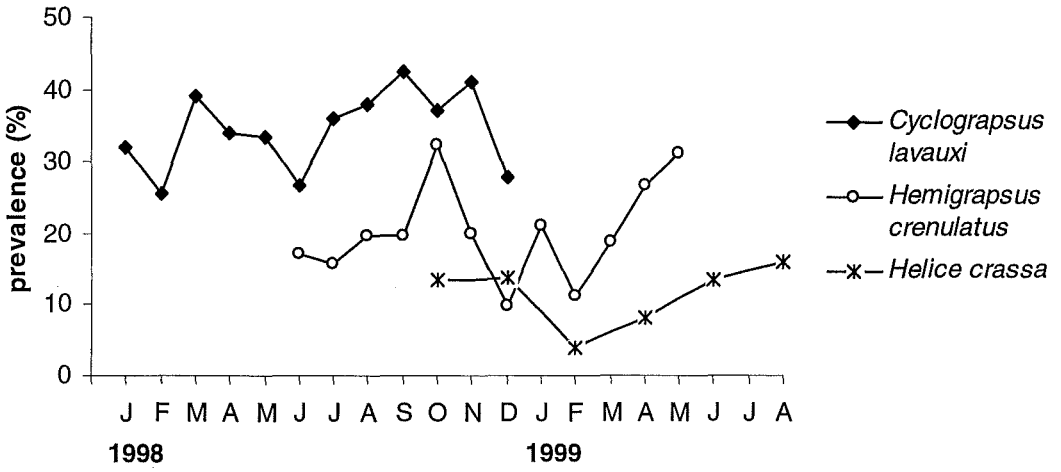
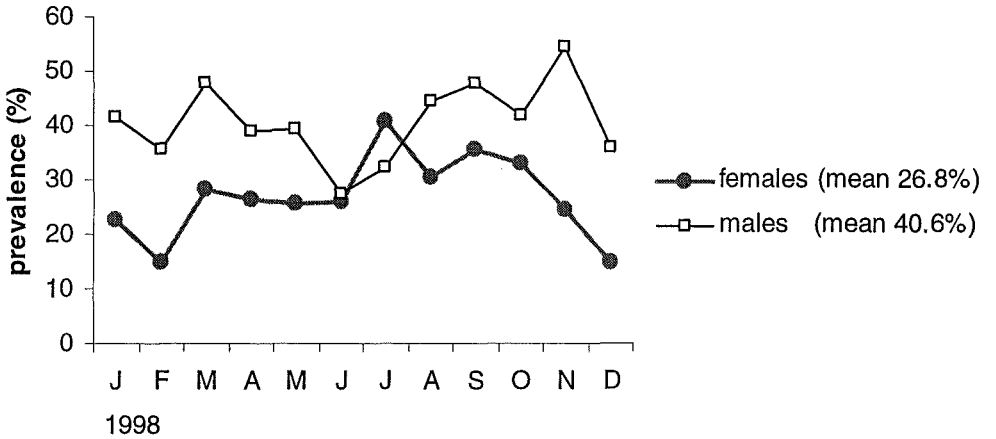
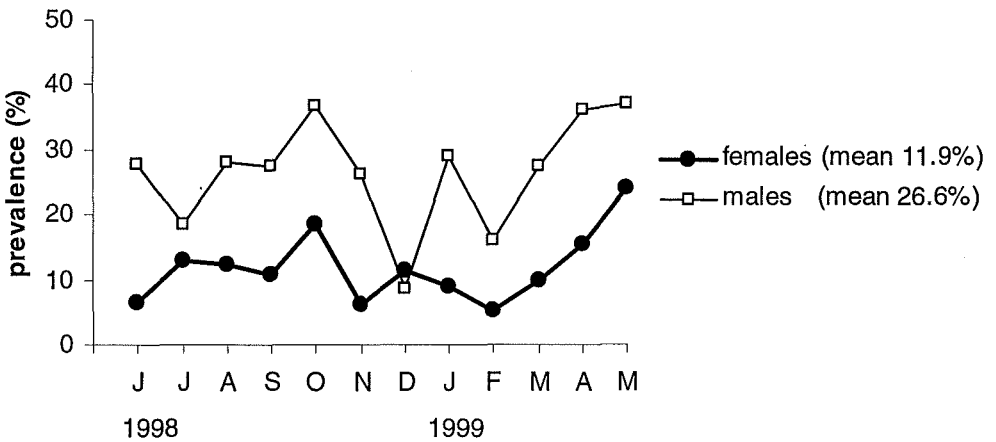


Figure 6.2 Seasonal prevalence of the parasite *Portunion* sp. in *Cyclograpsus lavauxi* (mean 34.4%) and *Hemigrapsus crenulatus* (mean 20.3%) from Governors Bay and *Helice crassa* (mean 11.6%) from the Avon-Heathcote Estuary in Canterbury, New Zealand.

A. *Cyclograpsus lavauxi*



B. *Hemigrapsus crenulatus*



C. *Helice crassa*

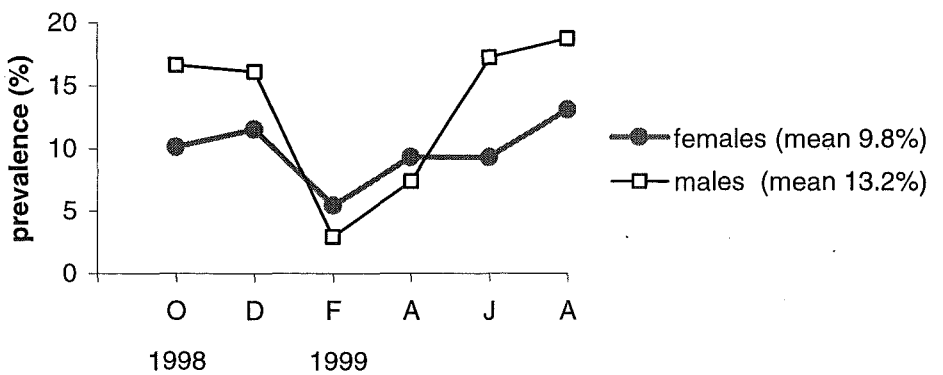
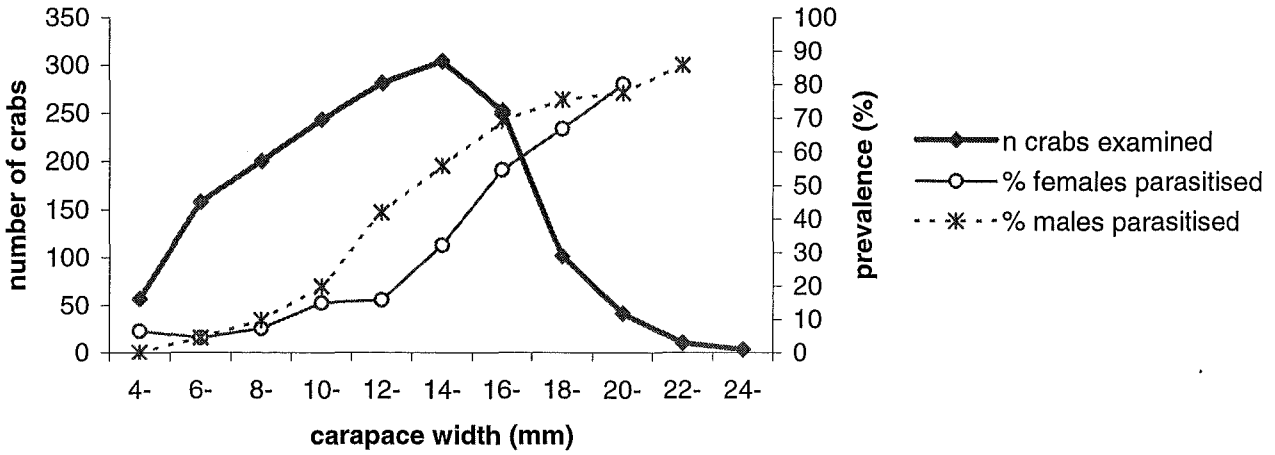
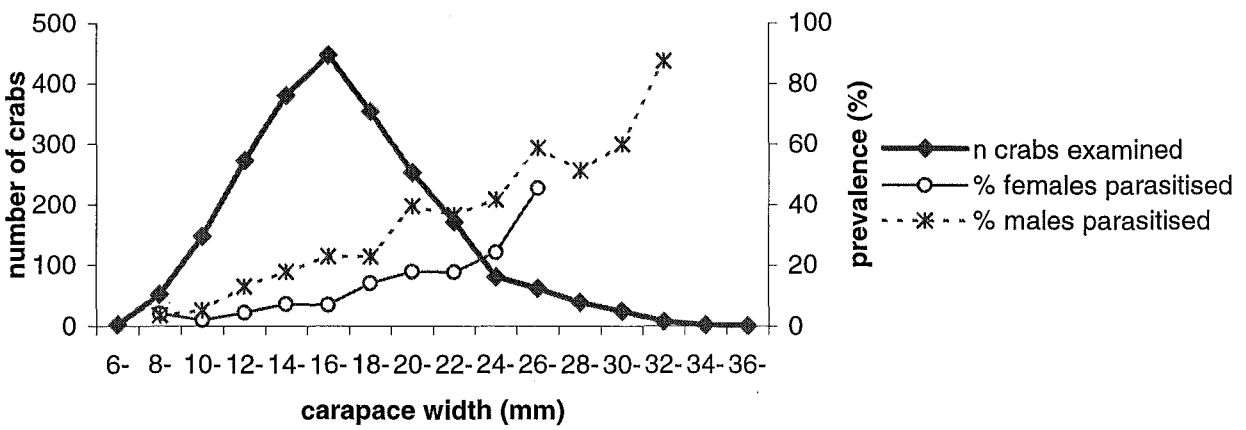


Figure 6.3 Seasonal prevalence of *Portunion* sp. in male and female: A. *Cyclograpsus lavauxi* collected from Lyttelton Harbour; B. *Hemigrapsus crenulatus* collected from Lyttelton Harbour; C. *Helice crassa* collected from the Avon-Heathcote Estuary.

A. *Cyclograpsus lavauxi*



B. *Hemigrapsus crenulatus*



C. *Helice crassa*

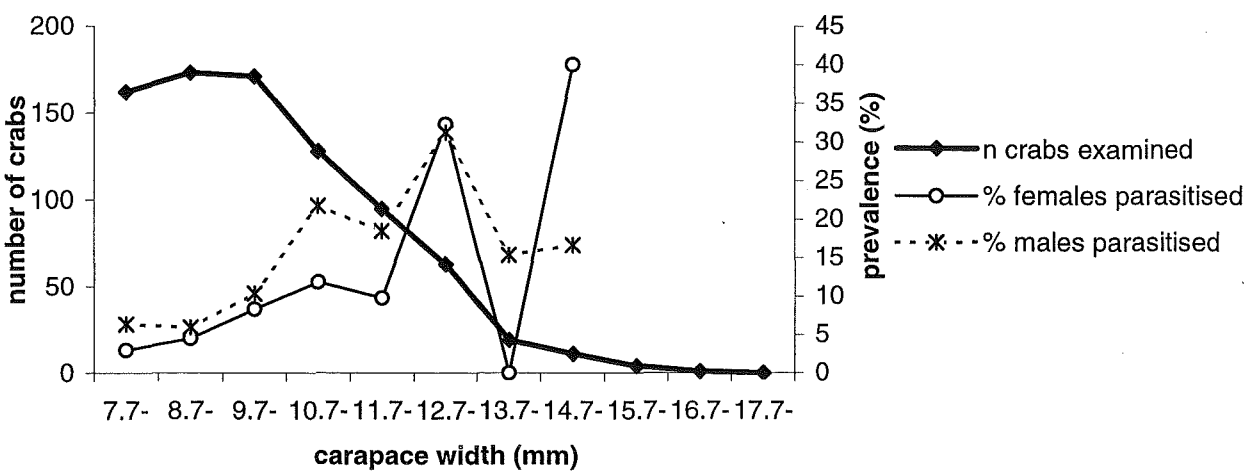
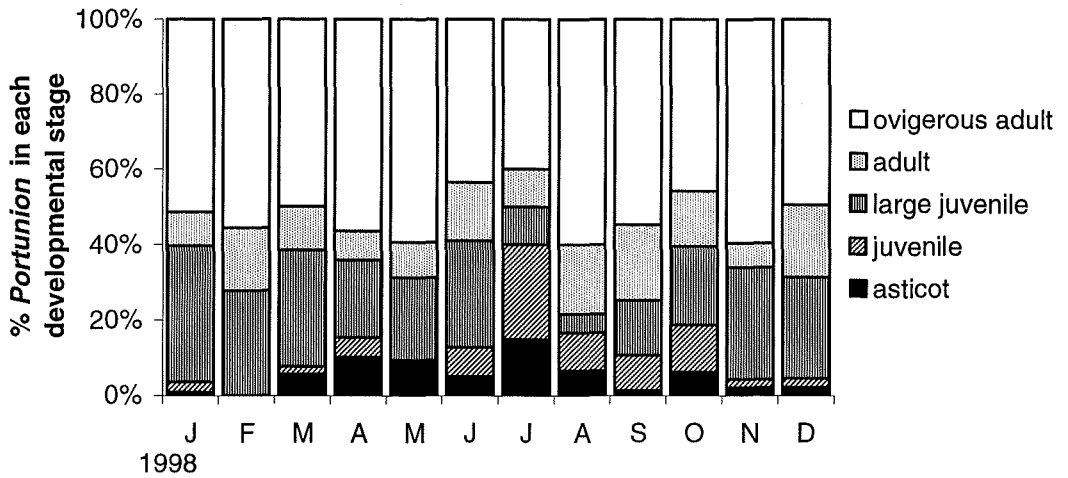
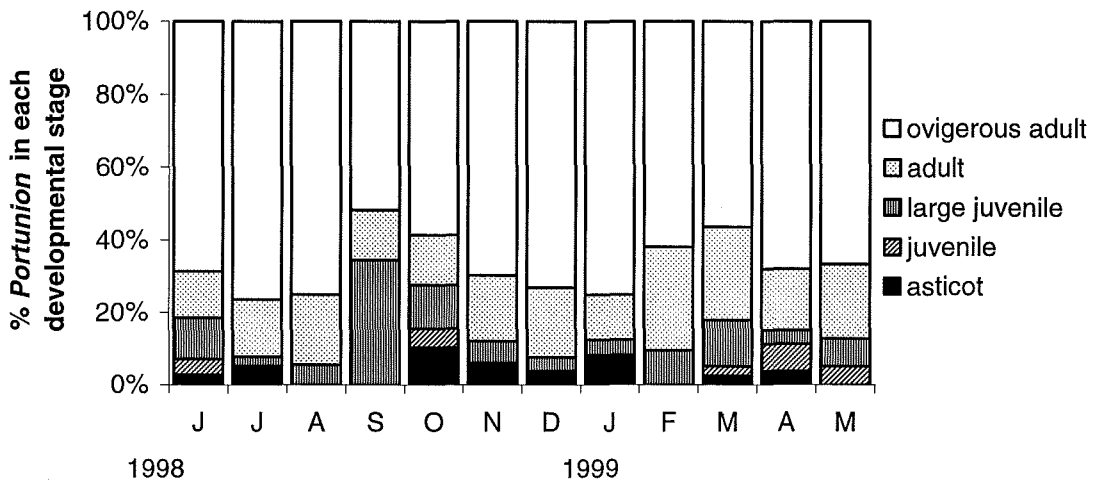


Figure 6.4 Prevalence of *Portunion* sp. in relation to male and female host size: A. *Cyclograpsus lavauxi*, B. *Hemigrapsus crenulatus*, and C. *Helice crassa*. Prevalence shown only if more than five crabs were collected in the size class. Number of crabs examined in each size class is also shown.

A. *Cyclograpsus lavauxi*



B. *Hemigrapsus crenulatus*



C. *Helice crassa*

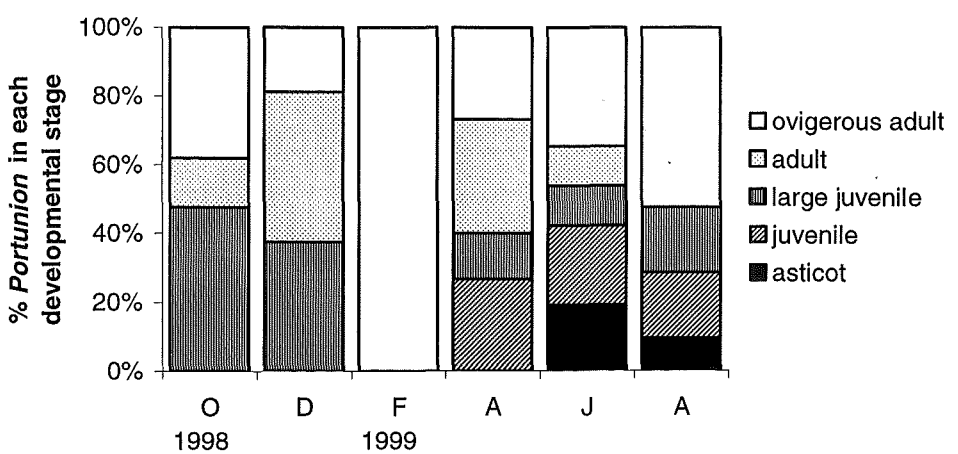
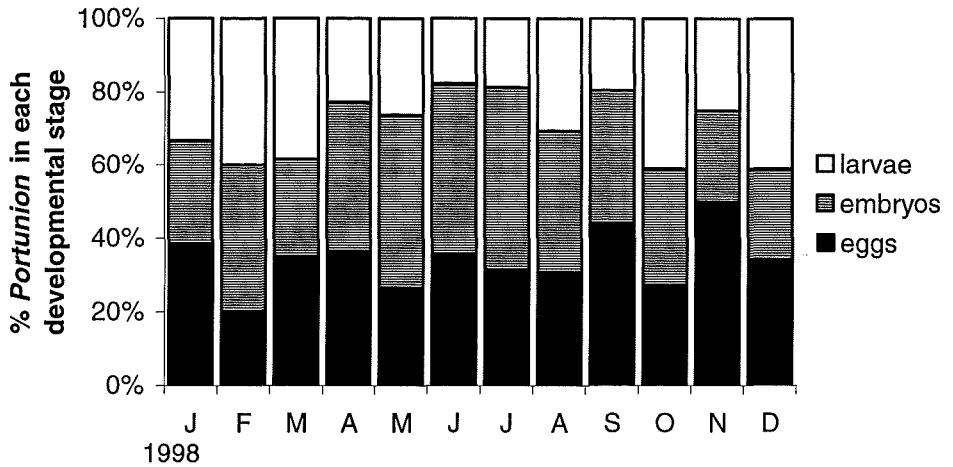
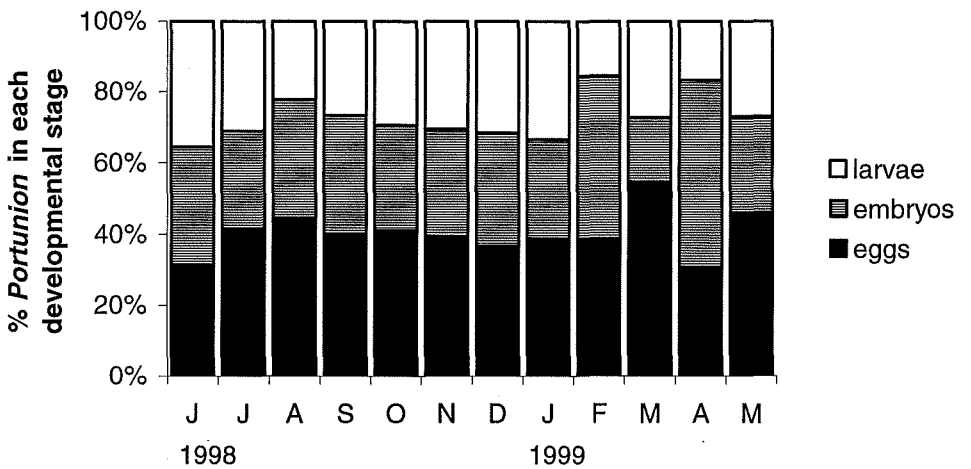


Figure 6.5 Developmental stages of female *Portunion* sp. in its hosts: A. *Cyclograpsus lavauxi*, (n = 650 female *Portunion* sp.); B. *Hemigrapsus crenulatus* (n = 466 female *Portunion* sp.); C. *Helice crassa* (n = 104 female *Portunion* sp.).

A. *Cyclograpsus lavauxi*



B. *Hemigrapsus crenulatus*



C. *Helice crassa*

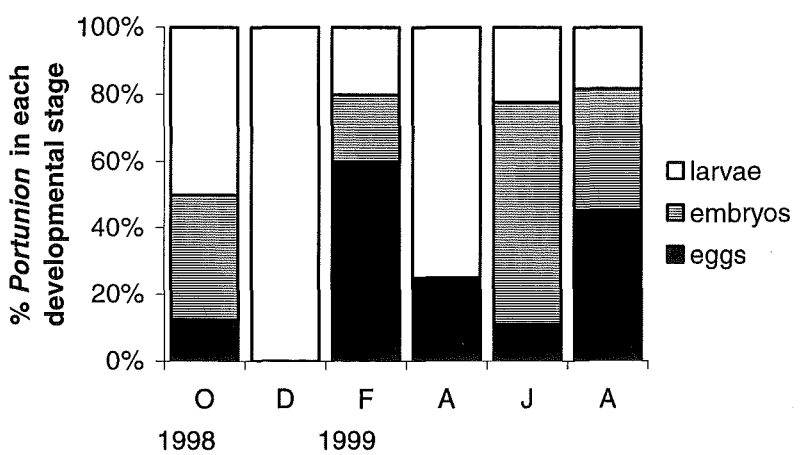


Figure 6.6 Contents of the brood pouch of ovigerous female *Portunion* sp. in its hosts: A. *Cyclograpsus lavauxi* (n = 338 ovigerous female *Portunion* sp.); B. *Hemigrapsus crenulatus* (n = 310 ovigerous female *Portunion* sp.); C. *Helice crassa* (n = 40 ovigerous female *Portunion* sp.).

7 Parasite-host associations of *Profilicollis* spp. (Acanthocephala: Polymorphidae) in two intertidal shore crabs (Brachyura: Grapsidae) from New Zealand

Abstract - The occurrence of cystacanths of *Profilicollis* spp. (Acanthocephala: Polymorphidae) was studied in relation to their intermediate hosts from two sites in Canterbury, South Island, New Zealand. *Profilicollis antarcticus* Zdzitowiecki and *Profilicollis novaezelandensis* Brockerhoff & Smales were found in the crabs *Hemigrapsus crenulatus* (Milne Edwards) and *Helice crassa* Dana (Brachyura: Grapsidae). *Profilicollis* spp. prevalence were similar for males and females and ranged from 4.2 to 45.2% for *Hemigrapsus crenulatus* and from 0% to 10.9% for *Helice crassa*. Parasite prevalence was highest in the autumn and winter months (April to August). There was no correlation between *Profilicollis* spp. prevalence or intensity and crab size. The results were compared with the occurrence of cystacanths of *Profilicollis* spp. in other decapod crustaceans and it appears that the prevalence of *Profilicollis* spp. cystacanths is highly variable. This variability likely reflects the abundance of the definitive hosts as well as other biotic and abiotic factors. The morphology of cystacanths of several species within the genus *Profilicollis* was found to be very similar in overall appearance. The number and arrangement of proboscis hooks are so similar for several species of *Profilicollis* that they cannot be used alone for identification.

7.1 Introduction

Acanthocephalans are endoparasites with a complex life cycle which involves an intermediate host, often a crustacean, a vertebrate definitive host and sometimes also a paratenic host (Schmidt, 1985). For example, the acanthocephalan parasite *Profilicollis botulus* inhabits the intestine of the eider duck (*Somateria mollissima*). The parasite's eggs, which are passed out with the faeces of the duck, are immediately infective upon ingestion by the portunid shore crab *Carcinus maenas*. In this intermediate host, they develop via an acanthella into the long-lived, infective cystacanths. Eiders then become parasitised by feeding on infected crabs (Rayski and Garden, 1961).

In general, the prevalence of a parasite in a particular host population is determined by biotic (i.e., ecological) and abiotic (i.e., climatic) factors which influence the probability of

contact between host and parasite (Poulin 1998). For example, parasite prevalence in a host population is likely to increase with increased exposure to the infective stages of the parasite. In addition, parasites can accumulate over time (Kennedy 1975). On the other hand, parasites can be selectively removed from a host population when the definitive host feeds selectively on infected individuals of the intermediate host. Prey selection by definitive hosts can be caused by parasite induced alteration of host behaviour. For example, isopods infected with the acanthocephalan *Plagiorhynchus cylindraceus* are over represented in the starling's, *Sturnus vulgaris*, diet due to behavioural alterations of the isopod (Moore 1983, 1984).

Few studies have been carried out in New Zealand on the distribution of adult acanthocephalans in their definitive hosts such as birds and terrestrial mammals (see checklists of McKenna 1997, 1998; Brockerhoff and Smales 2001). Even less is known about the occurrence of acanthocephalan larval stages (cystacanths) in New Zealand and about their effects on intermediate hosts (Brockerhoff and Smales 2001; Latham and Poulin 2001). A parasitological survey was therefore undertaken to determine the presence, seasonal variation and relationships with host gender and size of the acanthocephalans infecting two common intertidal crabs, *Hemigrapsus crenulatus* and *Helice crassa* (Brachyura: Grapsidae). Cystacanths of *Profilicollis* spp., a genus that has recently been resurrected because of its association with decapod crustaceans (Nickol et al. 1999), were found in both crabs. The prevalence of these cystacanths in crabs is discussed in relation to the presence of the definitive hosts. The occurrence of *Profilicollis* cystacanths in crabs from New Zealand and other parts of the world is compared. In addition, morphological features of cystacanths of the genus *Profilicollis* are compared to provide an overview of these developmental stages, which are usually ignored in comprehensive identification keys of adult descriptions. Common characters used for identifications are also discussed.

7.2 Materials and Methods

Crabs were collected at two sites in Canterbury in the South Island of New Zealand in 1998 and 1999. *Hemigrapsus crenulatus* was collected monthly from June 1998 to May 1999 using baited net traps at Governors Bay in Lyttelton Harbour (43° 38′ S, 172° 39′ E). All males collected during this period, a sub-sample of females collected from June to October, and all females collected from November 1998 to May 1999 were examined for acanthocephalan parasites (Table 7.1). All these crabs had a carapace width larger than 5.9 mm. *Helice crassa* was

collected every two months by hand, using quadrats (0.3 m x 0.3 m) every two metres along a 40 m transect, from October 1998 to August 1999 from the Avon-Heathcote Estuary, Christchurch (43° 33′ S, 172° 44′ E) (Table 7.1). All *Helice crassa* with a carapace width larger then 7.6 mm were dissected and examined for acanthocephalans.

Crabs were held in the laboratory for one to twelve weeks in tanks with circulating seawater at 12°C and fed blue mussels (*Mytilus edulis*) two or three times a week. Before dissection crabs were killed by placing them in a freezer at -15°C for about 1 hour. Acanthocephalan cystacanths were removed from the hemocoels of their hosts, and their numbers and positions together with the sizes (carapace width, CW) and gender of the hosts, were recorded. In addition, the internal organs of the hosts were examined under a binocular microscope at 160×. For identification, a random sub-sample of cystacanths was collected over the year (83 cystacanths from 69 *Hemigrapsus crenulatus*, 24 cystacanths from 23 *Helice crassa*). Cystacanths were placed in tap water until the proboscis everted, then fixed in alcohol-formalin-acetic acid (AFA) or 70% ethanol. Cystacanths used for light microscopy were dehydrated in 96% ethanol and cleared in beechwood creosote. Voucher specimens have been deposited in the National Museum, Wellington, New Zealand (*Profilicollis novaezelandensis*: NMNZ ZW 1496; *Profilicollis antarcticus*: NMNZ ZW 1497). Parasitological terms are used according to Bush et al. (1997). Statistics were calculated using the software SYSTAT 9.

7.3 Results

Identification and morphology of *Profilicollis* cystacanths

Two types of cystacanths belonging to the genus *Profilicollis* Meyer, 1931 were found in the haemocoel and lining the gut of the two crab species investigated. One was identified as *Profilicollis novaezelandensis* and the other as *Profilicollis antarcticus*. Morphological features of cystacanths of *P. antarcticus* and *P. novaezelandensis* are summarised in Tables 7.2 and 7.3. Five cystacanths of *P. antarcticus* from *Hemigrapsus crenulatus* from Chile were examined and compared with cystacanths of *P. antarcticus* from New Zealand. No morphological differences were found between the specimens from New Zealand and Chile. From the few cystacanth descriptions that are available from other species within the genus *Profilicollis*, it appears that cystacanths are very similar in respect to their overall morphology and size (Table 7.2). In addition, the number of proboscis hooks and their arrangement in longitudinal rows is variable and can overlap among the different species (Table 7.3). Although the proboscis armature and

morphology of cystacanths of *P. antarcticus* are very similar to *P. sphaerocephalus*, it was assumed that the former occurs in the crabs studied, because *P. antarcticus* was identified from two definitive hosts (South Island pied oystercatcher *Haematopus ostralegus finschi* Martens and bar-tailed godwit *Limosa lapponica* (Linnaeus)) from nearby sites (personal observation).

Profilicollis antarcticus and *P. novaezelandensis* were found at both sampling sites. However, 95.8% of the cystacanths in *Helice crassa* from the estuary were *P. antarcticus*, and 95.2% of the cystacanths in *Hemigrapsus crenulatus* from Lyttelton Harbour were *P. novaezelandensis* according to the sub-sample identified. No pathological effects of the parasites on their hosts were noted and the gonads of male and female crabs appeared to be normal.

Acanthocephalan cystacanths in *Hemigrapsus crenulatus*

Of the 1,970 *Hemigrapsus crenulatus* examined, 522 (26.5%)(27.8% of females, 25.8% of males) harboured cystacanths of *Profilicollis* spp. Monthly prevalence of cystacanths were similar for male and female crabs and ranged from 4.2% to 42.0% for females and from 13.1% to 45.2% for males (Fig. 7.1). Prevalence was lowest during November (the Southern Hemisphere spring)(Fig. 7.1). There was no correlation between parasite prevalence and crab gender (Pearson chi-square, $P = 0.33$, Fig. 7.1) or between parasite prevalence and crab size (linear regression, $R^2 = 0.003$; $P = 0.86$, Fig. 7.2). Most often one cystacanth was found per crab (57.0%), followed by two (23.2%), three (10.0%) and four (3.8%) cystacanths per host. Occasionally larger clusters of as many as 17 cystacanths per host were observed (Fig. 7.3). On average, 1.9 cystacanths were found in an infested crab. No correlation was found between parasite intensity (cystacanths per infected crab) and crab size (Fig. 7.4).

Acanthocephalan cystacanths in *Helice crassa*

Few *Helice crassa* were found to be infected (34 of 827, 4.1%) with cystacanths of *Profilicollis* spp. The overall parasite prevalence was not significantly different for male (3.4%) and female crabs (4.8%) (Pearson chi-square, $P = 0.31$). Monthly parasite prevalence was generally relatively low and varied between 0% and 7.3% for females and between 1.5% and 10.9% for males (Fig. 7.5). Lowest parasite prevalence for *Helice crassa* was found in December (early summer) (Fig. 7.5). There was no correlation between parasite prevalence and crab size (linear regression, $R^2 = 0.53$; $P = 0.1$, Fig. 7.6). Most infected crabs (94.1%) harboured one cystacanth. Only two of 34 infected crabs had multiple infestation, with 2 and 3 cystacanths respectively (mean intensity 1.1).

7.4 Discussion

Identification and morphology of *Profilicollis* cystacanths

The cystacanths in the present study were identified as *P. antarcticus* and *P. novaezelandensis*. However, cystacanths of all species of *Profilicollis* were found to be very similar in respect to their overall morphology and size. Combined with the absence of reproductive structures or lack of information on these, species identification at the cystacanth level can be difficult, unless the life cycle is tested experimentally and the adults can subsequently be examined for identification. More information is therefore necessary on cystacanth structures which might allow better identification. Denny (1969), for example, was able to separate cystacanths of some *Polymorphus* species (*Po. marilis*, *Po. contortus*, *Po. paradoxus*) by differences in the size of the largest proboscis hook. The occurrence of adult Acanthocephala at, or near, the sampling site where larval stages (cystacanths) were found was used in the present study as an additional indicator for the identification of cystacanths.

Cystacanths of both *Profilicollis* species occurred in both crab species studied. However, *P. antarcticus* was dominant at the estuary and *P. novaezelandensis* was dominant in Lyttelton Harbour. This could be due to differences in abundance of the bird species that act as definitive hosts or levels of infection of individual birds at the two sampling sites.

Parasite prevalence and seasonality

Cystacanths of *Profilicollis* spp. were much more common in *Hemigrapsus crenulatus* (26.3%) compared to *Helice crassa* (4.1%). This could be due to the fact that the crabs were sampled at two locations with different biotic and abiotic conditions. For example, there could be differences in the abundance of the definitive hosts, which determines the availability of infective stages (eggs). In addition, the survivorship of the parasite eggs could be differentially affected by the lower salinity of the estuary, where *Helice crassa* were collected, compared to the marine harbour where *Hemigrapsus crenulatus* came from. A literature survey of the occurrence of cystacanths of other species of *Profilicollis* revealed that prevalence can vary between 1 and 100%, depending on the parasite and host species (Table 7.4). Most of the data available for prevalence of *Profilicollis* cystacanths were obtained from studies with single observations and with small sample sizes (Table 7.4). For a few species, aspects of the life cycle and distribution within its intermediate host have been studied in more detail, for example, *P. botulus* (Van Cleave) (Liat & Pike 1980; Thompson 1985a, b), *P. altmani* (Perry) (Karl 1967, as

cited in Nickol et al. 1999), and *P. chasmagnathi* (Holcman-Spector, Mañé-Garzón & Dei-Cas) (Martorelli 1989).

In the present study, *Profilicollis* spp. cystacanth abundance peaked in autumn and winter (April to August). Adults of *P. antarcticus* and *P. novaezelandensis* have been found in the South Island pied oystercatcher and the bar tailed godwit in New Zealand (personal observations). As no seasonal data are available concerning the prevalence of *Profilicollis* spp. in these birds, the abundance of cystacanths and adults of *Profilicollis* cannot be compared directly.

However, during autumn and winter, the probability of parasite transfer (from crab to bird) would be relatively high because of the higher parasite prevalence in the crabs. This coincides with the arrival of the South Island pied oystercatchers, which after breeding inland, migrate back to their winter sites which are the estuaries and mudflats along the coast (Baker 1973, 1974). Here, the South Island pied oystercatchers feed on intertidal crabs such as *Helice crassa* as well as on bivalve molluscs, which is their main food resource (Baker 1974). The only estimates for development times are about two to three months from ingestion of the infective egg to the cystacanth for *P. botulus* in *C. maenas* (Rayski & Garden 1961; Thompson 1985a) and a few weeks from maturation to egg release in the eider duck *S. mollisima* (Linnaeus) for *P. botulus* (Liat & Pike 1980). This suggests that during the course of the winter, infected oystercatchers may release acanthocephalan eggs, which will be eaten by the crabs. The lowest parasite prevalence in crabs were observed in early summer, which might be due to the fact that parasitised crabs have been selectively removed out of the population during winter by the definitive hosts (see selective removal of crabs below). A month after the departure of the oystercatchers (around October), parasite prevalence in crabs increases again. This indicates that crabs are still exposed to the infective stages and that other hosts (see below) also influence cystacanth prevalence in the New Zealand crabs.

The bar-tailed godwit is also likely to include intertidal crabs such as *Hemigrapsus* in its diet as has been reported for the closely related *Limosa fedoa* (see Gratto-Trevor 2000). However, the bar-tailed godwit is mainly a summer visitor to New Zealand (Higgins & Davies 1996). It arrives, for example, at the Avon-Heathcote estuary in late September and departs in late March to early April, leaving only a small over-wintering population behind (Pierre 1994). As parasite prevalence was relatively low during summer, it appears that godwits are less likely than oystercatchers to acquire cystacanths from the crabs.

Apart from the two known records of *Profilicollis*, other birds such as the black-backed gull *Larus dominicanus*, the introduced (European) starling *S. vulgaris*, and the New Zealand

kingfisher *Halcyon sancta*, could potentially be hosts for *Profilicollis* in New Zealand. The black-backed gull has been reported as a definitive host for *P. antarcticus* in Chile (Haye & Ojeda 1998). The black backed gull is absent form the Avon-Heathcote estuary during spring and early summer. Most of them return in February and increase in number in winter (Owen 1992). The starling has been observed feeding on *Helice crassa* (personal observations) and the New Zealand kingfisher feeds almost exclusively on *Helice crassa* (Hayes 1989). New Zealand kingfishers change their distribution seasonally, increasing in number on the coast and on estuaries in winter (Taylor 1966; Ralph & Ralph 1977). This would again coincide with higher prevalence of cystacanth in crabs during winter. The involvement of several definitive hosts would not be unusual as this seems to be the case in several other species of *Profilicollis* such as *P. botulus* and *P. sphaerocephalus* (see Amin 1992).

Parasite prevalence and intensity versus host gender and size

Parasite prevalence in male and female crabs was not significantly different in the present study. The same was found for *P. botulus* in *C. maenas* (Liat & Pike 1980; Thompson 1985a) and *P. chasmagnathi* in *Cyrtograpsus angulatus* (Martorelli 1989).

In the present study, no correlation was found between parasite prevalence and host size or between parasite intensity and host size. This result was somewhat unexpected for the following reasons. First, juveniles and adults of both species, *Hemigrapsus crenulatus* and *Helice crassa,* are found in the same habitat (McLay 1988) and are most likely to be exposed at a similar rate to the infective eggs. Second, it has been estimated that the life spans of *Hemigrapsus crenulatus* and *Helice crassa* are about 3.5 to 5 years and 5 to 6 years, respectively (McLay 1988). This is enough time to expect accumulation of cystacanths in older and, therefore, larger crabs. Early developmental stages of *Profilicollis* were found in all size classes in the current study; however, it is not known whether a certain size class of crabs is more or less likely to become infected.

Some studies of *Profilicollis* cystacanths in decapods found a correlation between parasite prevalence and host size, and parasite intensity and host size (Liat & Pike 1980; Thompson, 1985a; Latham & Poulin 2001), but others did not (Brattey & Campbell 1985), or found only a correlation between parasite prevalence and host size (Martorelli 1989). The prevalence and intensity of cystacanths were shown to increase with increasing host size in the case of *P. botulus* and the green shore crab *C. maenas* (Liat & Pike 1980; Thompson 1985a). The life span for *C. maenas* is estimated to be 3 - 4 years in Britain and 5 - 6 years in central Maine (Berril 1982), and, therefore, similar to *Hemigrapsus crenulatus* and *Helice crassa* in New Zealand. Other parameters such as crab size and amount of food intake of *C. maenas* are different compared to

Hemigrapsus crenulatus and *Helice crassa.* For example, *C. maenas* has a larger maximum carapace width (up to 70 mm; Liat & Pike 1980) compared to *Hemigrapsus crenulatus* and *Helice crassa* (maximum carapace widths of about 17 mm and 28 mm, respectively) and, therefore, must consume more food, which increases the probability of ingestion of infective stages. The overall parasite prevalence in *C. maenas* (32% in Liat & Pike 1980; 36.7% in Thompson 1985a) was higher compared to *Hemigrapsus crenulatus* (26%) and *Helice crassa* (4%), which might also indicate a higher exposure rate of *C. maenas* to parasitism. However, the general life patterns of the three crab species are very similar and the general trend of increased parasite prevalence over time, i.e., with age and size, would be expected for all of them. This trend would also be expected for the grapsid crab *C. angulatus.* Indeed, a correlation was found between *P. chasmagnathi* prevalence and crab size, but not between parasite intensity and crab size, even though parasite prevalence in general is very high (68%; see Martorelli 1989).

No correlation between parasite prevalence and host size nor between parasite intensity and host size was found for *P. botulus* and the lobster *Homarus americanus* (Brattey & Campbell 1985). As *H. americanus* commonly migrate during their life (Campbell & Stasko 1985), small and large lobster mostly inhabit different habitats (inshore and offshore, respectively). Young lobsters near the shore are exposed to the eggs or cystacanths, the latter while feeding on infected inshore crabs as was suggested by Brattey and Campbell (1985). Once the lobster migrate offshore, they do not acquire further infections.

Parasite prevalence can be partially controlled by the selective removal of infected crabs by the definitive hosts. For example, field and laboratory experiments have been carried out on behavioural alteration of *Hemigrapsus crenulatus* by cystacanths of *P. antarcticus* in Chile and New Zealand (Pulgar et al. 1995; Haye & Ojeda 1998; Latham & Poulin 2001). In the field, cystacanths did not seem to induce significant behavioural alterations to an artificial threat and the use of habitats (Pulgar et al. 1995; Latham & Poulin 2001). However, in the laboratory, parasitised crabs were more active and had higher metabolic rates than uninfected crabs (Haye & Ojeda 1998), which would make them more prone to predation. If birds feed selectively on parasitised crabs as well as a particular size group, they could have a profound effect on parasite prevalence in relation to the size of the crab host.

Interestingly, in the case of *C. maenas,* larger crabs were less readily eaten by eider ducks in feeding trials, due to the fact that larger crabs are less easily captured (Liat & Pike 1980). Observation of a natural eider duck population also revealed that larger crabs were less frequently eaten (Thompson 1985b). Large *H. americanus* are also not eaten by eiders and

scoters, *Melanitta* spp., which are the final hosts of *P. botulus* (see Brattey & Campbell 1985). From a parasite's point of view, developing or accumulating in large *C. maenas* or *H. americanus* is a disadvantage, as the transfer to the definitive host is reduced and the life cycle is unlikely to be completed.

In summary, it appears that the abundance and feeding behaviour of the definitive hosts together with the activity patterns of the crabs (behaviour of parasitised versus not parasitised crabs) have an impact on the prevalence and seasonal changes of the cystacanths of *Profilicollis* in crabs. However, the extent of these factors has yet to be established. In addition, other biotic and abiotic factors, such as the possibility of increased defence capability of the crab with age or the effect of temperature or salinity changes on egg survival could also have an impact on parasite prevalence.

Table 7.1 Number of male and female *Hemigrapsus crenulatus* and *Helice crassa* dissected in 1998 and 1999.

Date of collection		Total numbers examined (males/females)	
		Hemigrapsus crenulatus	*Helice crassa*
1998	Jun	252 (190 / 62)	
	Jul	150 (114 / 36)	
	Aug	120 (85 / 35)	
	Sept	113 (73 / 40)	
	Oct	132 (117 / 15)	147 (69 / 78)
	Nov	155 (107 / 48)	
	Dec	262 (140 / 122)	114 (52 / 62)
1999	Jan	114 (69 / 45)	
	Feb	176 (99 / 77)	123 (55 / 68)
	Mar	198 (98 / 100)	
	Apr	184 (100 / 84)	156 (75 / 81)
	May	114 (62 / 52)	
	Jun		162 (75 / 87)
	Jul		
	Aug		125 (61 / 64)
	Total	1,970 (1,254 / 716)	827 (387 / 440)

Table 7.2 Comparison of morphological features of cystacanths of *Profilicollis*. Measurements are given in micrometers as a mean and the range in parentheses if available. Data obtained from other sources are referred to by a symbol following the species name. No data were available on cystacanths of *P. arcticus* and *P. formosus*.

Species	*P. altmani* [1]	*P. antarcticus*[2]		*P. botulus* [3]	*P. bullocki* [4]
Gender	Combined	Male	Female	Combined	Combined
Proboscis length	571 (518-648)	425 (398-455)	522 (505-537)	590 (510-632)	550 (450-600)
Proboscis width	269 (230-364)	333 (316-347)	408 (392-417)	318 (347-408)	330 (260-390)
Proboscis receptacle length	1,868 (1,718-2,016)	1,548 (1,495-1,639)	2,062 (1,957-2,259)	1,342 (1,204-1,530)	2,380 (1,870-3,000)
Proboscis receptacle width	N/A	242 (225-269)	266 (247-285)	295 (204-347)	N/A
Leminisci length	1,251 (1,037-1,440)	907*	819 (811-827)	N/A	1,550 (1,300-1,900)
Neck length	1,512 (1,001-1,740)	827 (716-955)	1,088 (891-1225)	673 (449-796)	N/A
Neck width	406 (336-470)	358 (291-448)	373 (316-429)	338 (245-653)	N/A
Anterior trunk length	1,253 (1,219-1,344)	981 (827-1193)	1,260 (1,130-1,400)	947 (551-1224)	N/A
Anterior trunk width	491 (394-576)	564 (537-587)	648 (632-701)	577 (428-714)	N/A
Mid-trunk length	1,613 (1,248-2,285)	not specified	not specified	not specified	not specified
Mid-trunk width	663 (624-845)	not specified	not specified	not specified	not specified
Posterior trunk length	683 (650-701)	1,156 (1,114-1,193)	1,384 (1,225-1,368)	1,279 (1,122-1,469)	N/A
Posterior trunk width	151 (148-154)	640 (568-726)	733 (682-764)	733 (642-836)	(890-1130)
Anterior testis length		139 (138-141)		N/A	N/A
Anterior testis width		110 (102-117)		N/A	N/A
Posterior testis length		144 (131-157)		N/A	N/A
Posterior testis width		106 (90-120)		N/A	N/A
Testis length **	134-139	see above		N/A	N/A
Testis width **	101-110	see above		N/A	N/A
Cement glands		not observed		N/A	N/A
Copulatory bursa length		N/A		N/A	N/A
Copulatory bursa width		N/A		N/A	N/A
Overall length		3,390 (3,055-3,796)	4,181 (3,751-4,530)	3,488	(5,500-7,500)

[1] Nickol et al. 2002, [2] Brockerhoff and Smales, 2001; [3] Ching, 1989; [4] Mateo et al., 1984; ** anterior and posterior testis combined; N/A, data not available.

Table 7.2 Continued. Comparison of morphological features of cystacanths of *Profilicollis*.

Species	*P. chasmagnathi* [5]		*P. major* [6, #]	*P. novaezelandensis*[1]		*P. sphaerocephalus* [7]	
Gender	Male	Female	?	Male	Female	Male	Female
Proboscis length	N/A	(440-680)	560	581 (515-665)	611 (578-665)	467 (408-577)	516 (377-658)
Proboscis width	N/A	(260-500)	280	388 (325-436)	440 (396-475)	327 (282-375)	371 (269-445)
Proboscis receptacle length	(2,000-2,550)	(2,220-3,450)	1,380	1,533 (1,280-1,680)	1,618 (1,440-1,780)	1,578 (1,703-2,419)	1894 (854-3096)
Proboscis receptacle width	(150-270)	N/A	400	291 (268-320)	316 (268-364)	228 (192-385)	244 (115-329)
Leminisci length	(1,390-1,940)	2,220*	N/A	N/A	N/A	2,903*	2,593 (1,780-3,270)
Neck length	(550-1,440)	(1,110-1670)	580	839 (610-960)	836 (660-860)	1042 (832-1316)	1,084*
Neck width	(220-300)	(280-420)	N/A	396 (341-451)	450 (396-515)	296 (238-369)	310*
Anterior trunk length	(1,170-1,610)	(1,220-2,180)	N/A	1,135 (1,040-1,260)	1,154 (1,100-1,240)	1,202 (948-1,364)	1,360 (1,006-1,606)
Anterior trunk width	(500-670)	(610-940)	N/A	553 (475-665)	610 (540-689)	544 (368-716)	583 (416-774)
Posterior trunk length	(1,270-2,000)	(1,520-2,880)	N/A	1,278 (1,160-1,460)	1,384 (1,280-1,460)	1,339 (784-1,664)	1,609 (1,316-2,012)
Posterior trunk width	(560-830)	(780-1,220)	N/A	687 (594-820)	754 (657-816)	866 (716-1,374)	862 (726-1006)
Anterior testis length	N/A		N/A	190 (164-248)		151 (112-223)	
Anterior testis width	N/A		N/A	111 (94-128)		98 (72-131)	
Posterior testis length	N/A		N/A	183 (154-240)		153 (132-223	
Posterior testis width	N/A		N/A	112 (98-138)		103 (79-138)	
Testis length [**]	(140-260)		N/A	see above		see above	
Testis width [**]	(100-120)		N/A	see above		see above	
Cement glands	present		N/A	not observed		not observed	
Copulatory bursa length	(530-740)		N/A	not observed		N/A	
Copulatory bursa width	(170-300)		N/A	not observed		N/A	
Overall length	(3,980-5,240)	(5,170-6,780)	N/A	3,851 (3,496-4,305)	3,985 (3,718-4,254)	4,050	4,569

[1]Brockerhoff and Smales, 2001; [5] Holcman-Spector et al., 1977a; [6] Schmidt and MacLean 1978; [#] n = 1; [7] Pichelin et al., 1999; [**] anterior and posterior testis combined; N/A, data not available.

Table 7.3 Number and arrangement of proboscis hooks in species of *Profilicollis.*

Species of *Profilicollis* *	**Proboscis hooks** Number of longitudinal rows	Number of hooks per longitudinal row
P. formosus (Schmidt & Kuntz, 1967)	12 - 15	8 (7 - 9)
P. novaezelandensis Brockerhoff & Smales, 2001	14 - 16	7 - 8
P. botulus (Van Cleave, 1916)	16 - 18 (20)	7 - 8
P. chasmagnathi (Holcman-Spector, Mañé-Garzón & Dei-Cas, 1977)	16 - 20 †	6 - 8 ‡
	16 - 18 ‡	8 - 9 †,§
	18 - 20 §	
P. major (Lundström, 1942)	16 - 20	7
P. sphaerocephalus (Bremser in Rudolphi, 1819)	17 - 21 ‖	7 - 8 ‖
	23 #	10 #
	26 - 28 ¶	12 - 14 ¶
P. antarcticus Zdzitowiecki, 1985	18 - 22	7 - 8/9
P. arcticus (Van Cleave, 1920)	22	7 - 8
P. altmani (Perry, 1942)	25 - 30 (28)	9 - 12 (11)
(*P. texensis* (Webster, 1948)) ††	22 - 24	8 - 10
(*P. kenti* (Van Cleave, 1947)) ††	27	10 - 11
P. bullocki (Mateo, Córdova & Guzmán, 1982)	27 - 33	13 - 15

Species sorted by increasing number of longitudinal rows of proboscis hooks; † Holcman-Spector et al. 1977b; ‡Vizcaíno 1989; § Martorelli 1989; ‖ Johnston and Edmond 1947; # Travassos 1926 as cited in Johnston & Edmond 1947; ¶ Marval, 1905 as cited in Johnston & Edmond 1947; †† synonyms of *P. altmani* (Nickol et al. 2002).

Table 7.4 Prevalence of cystacanths of *Profilicollis* spp. (Polymorphidae) in decapod hosts.

Species of *Profilicollis*	Decapod intermediate host	Prevalence (%)	Number of crabs examined	Locality	Reference
P. altmani	*Emerita analoga*	90	5,900	California	Karl (1967) cit. in Nickol et al.,1999
P. antarcticus	*Hemigrapsus crenulatus*	49	284	Chile	Pulgar et al., 1995
P. botulus	*Cancer irroratus*	0 - 12	128	Eastern Canada	Brattey et al., 1985
P. botulus	*Carcinus maenas*	0 - 10	119	Eastern Canada	Brattey et al., 1985
P. botulus	*Carcinus maenas*	43	68	Scotland	Rayski and Garden, 1961
P. botulus	*Carcinus maenas*	32	2,238	Scotland	Liat and Pike, 1980
P. botulus	*Carcinus maenas*	37	1,939	Scotland	Thompson, 1985a
P. botulus	*Hemigrapsus oregonensis*	9 - 62	734	Western Canada	Ching, 1989
P. botulus	*Homarus americanus*	1 - 10	885	Eastern Canada	Brattey and Campbell, 1985
P. botulus	*Hyas araneus*	100	N/A	Barents Sea	Uspenskaja (1960) cit. in Ching, 1989
P. botulus	*Hyas araneus*	80	N/A	Scotland	Nickol et al., 1999
P. botulus	*Necora puber*	24	N/A	Scotland	Nickol et al., 1999
P. botulus	*Pagurus pubescens*	12	N/A	Barents Sea	Uspenskaja (1960) cit. in Ching, 1989
P. bullocki	*Emerita analoga*	10 - 50	577	Peru	Oliva et al., 1992
P. chasmagnathi	*Chasmagnathus granulata*	41	1,015	Uruguay	Holcman-Spector et al., 1977a
P. chasmagnathi	*Cyrtograpsus angulatus*	68	138	Argentina	Martorelli, 1989
P. kenti[1]	*Emerita analoga*	86	109	Oregon	Reish, 1950
P. major	*Cancer irroratus*	20 - 37	71	Maine	Schmidt and MacLean, 1978
P. sphaerocephalus	*Paragrapsus laevis*	33 - 89	12	E-S. Australia	Pichelin et al., 1998
P. sphaerocephalus	*Nectocarcinus intergrifrons*	4	23	E-S. Australia	Pichelin et al., 1998
P. sphaerocephalus	*Brachynotus spinosus*	15 - 40	18	E-S. Australia	Pichelin et al., 1998
P. sphaerocephalus	*Cyclograpsus granulosus*	9 - 100	50	E-S. Australia	Pichelin et al., 1998
P. sphaerocephalus	*Paragrapsus quadridentatus*	14	22	E-S. Australia	Pichelin et al., 1998
P. sphaerocephalus	*Paragrapsus gaimardii*	3 - 50	131	E-S. Australia	Pichelin et al., 1998
Profilicollis spp.	*Macrophthalmus hirtipes*	94	250	New Zealand	Latham & Poulin, 2001
Profilicollis spp.	*Hemigrapsus crenulatus*	26	1,779	New Zealand	present study
Profilicollis spp.	*Helice crassa*	4	827	New Zealand	present study

[1] synonym of *P. altmani* (Nickol et al. 2002)

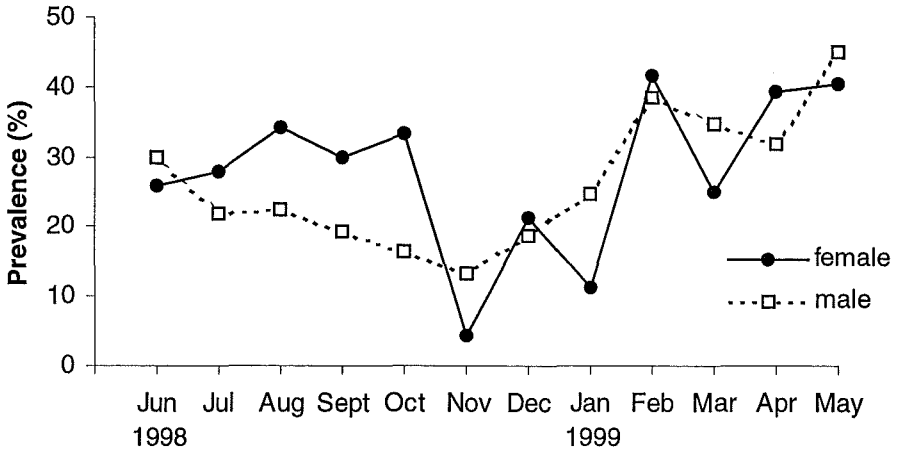


Figure 7.1 Prevalence of *Profilicollis* spp. cystacanths in male and female *Hemigrapsus crenulatus* over a one year period from June 1998 to May 1999.

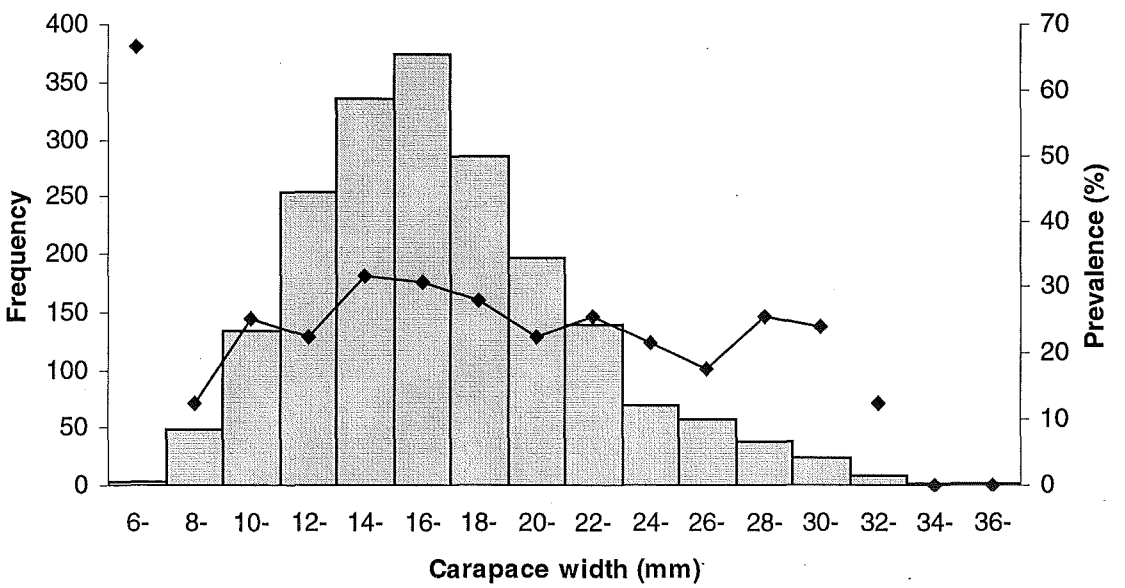


Figure 7.2 Size-frequency distribution of *Hemigrapsus crenulatus* collected from June 1998 to May 1999 (columns) and prevalence of *Profilicollis* spp. cystacanths (line). Diamonds not connected by line indicate size range with sample size < 9.

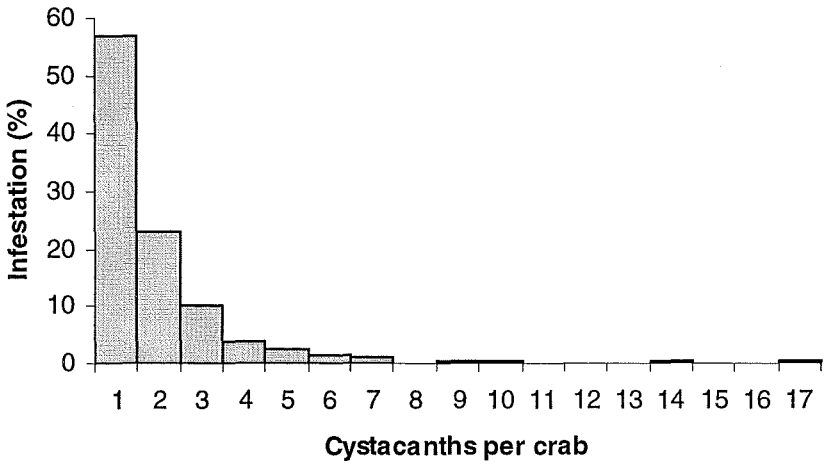


Figure 7.3 Prevalence of single and multiple infestation by *Profilicollis* spp. in *Hemigrapsus crenulatus*.

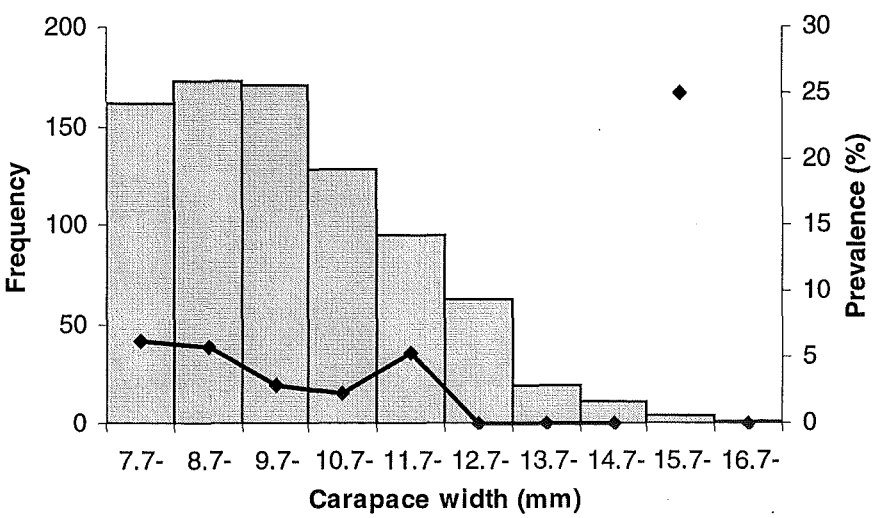


Figure 7.4 Prevalence of single and multiple infestation by *Profilicollis* spp. in different size classes of *Hemigrapsus crenulatus*. Diamond not connected by line indicate size range with sample size < 9.

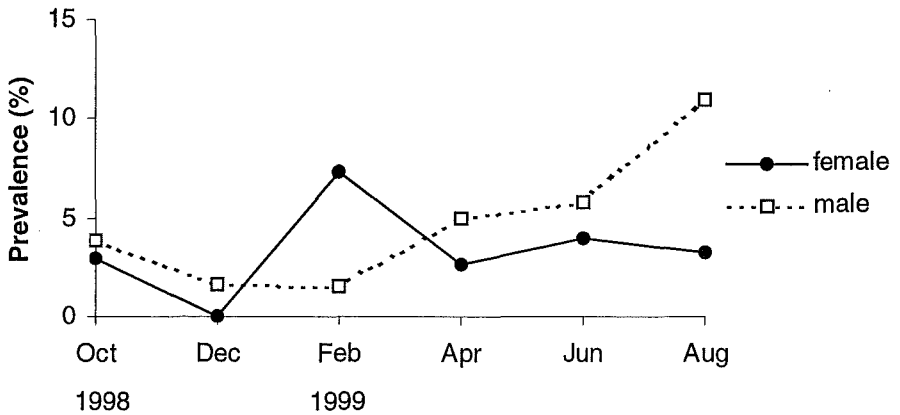


Figure 7.5 Prevalence of *Profilicollis* spp. cystacanths in male and female *Helice crassa* from October 1998 to August 1999.

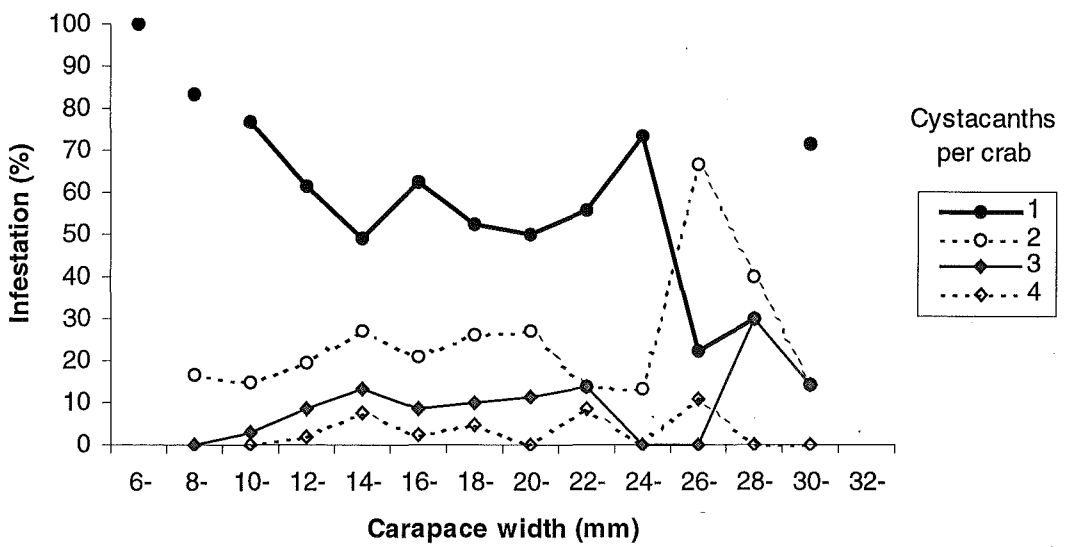


Figure 7.6 Size-frequency distribution of *Helice crassa* collected from October 1998 to August 1999 (columns) and prevalence of *Profilicollis* spp. cystacanths (line). Filled circles not connected by line indicate size range with sample size < 9.

8 Conclusions

The present study documented the mating strategies of four grapsid crabs (*Cyclograpsus lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus*, and *H. sexdentatus*) with emphasis on mating behaviour, duration of female sexual receptivity, male-male competition, and sperm competition. In addition, the parasite fauna of these four grapsid species was examined and the effects of parasites on host reproduction and host-parasite associations investigated.

Mating strategies in grapsid crabs: mating behaviour and factors influencing female receptivity, male-male competition, and sperm competition

Mating behaviour and mating systems

The general mating behaviour of the four grapsid species (*C. lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus*, *H. sexdentatus*) showed many similarities but differed in several important aspects that strongly influenced the operational sex ratio and the intensity of sperm competition (Table 8.1). In all four species investigated here, mating occurred during the intermoult when females developed mobile gonopore opercula and became sexually receptive. This was one of the two alternatives common in Grapsidae: mating by females can either be restricted to certain times because of immobile gonopore opercula or with one example (*Pachygrapsus crassipes*) after moulting, or always be possible because females have either no opercula, a reduced opercula, or a well-developed opercula that is always mobile (Chapter 5, Table 5.1). Consequently, it is likely that the operational sex ratio differs greatly among Grapsidae with different gonopore structures.

Females mated multiple times usually with several males in the few days prior to oviposition. Only female *Helice crassa* stayed receptive for two to three weeks after oviposition, and although they mated on average 24 times prior to oviposition (Table 8.1), they continued to mate afterwards. Mating immediately after egg-laying has been previously reported for the spider crab *Chionoecetes opilio* (Sainte-Marie et al. 1997) and the ocypodid crab *Macrophthalmus hirtipes* (Jennings et al. 2000). It has been suggested that it might be beneficial from a male's point of view, to increase their probability of paternity in subsequent broods (Sainte-Marie et al. 1997, 1999) and as a strategy used by low-quality males, who do not gain access to non-ovigerous or higher quality females, to increase their chances to fertilise subsequent broods (Sainte-Marie et al. 1999, Jennings et al. 2000). However, it needs to be

established what the costs and benefits for females are because only a few females have the strategy of staying receptive after egg laying.

Female grapsids retain sperm after moulting and are able to store sperm between the breeding seasons, and can therefore fertilise a new brood without mating again (Chapters 2 – 5). Multiple matings leading to a large sperm store might therefore be beneficial for the female for future broods. However, it was shown for *Hemigrapsus crenulatus* that females mate before each brood (Chapter 4) regardless of the amount of sperm stored and might therefore not use the sperm stored from the previous mating season (depending on the way sperm is stored in the spermathecae, i.e., whether sperm mixing or sperm layering occurs; see below). Several suggestions have been made why females in general might benefit from mating with several males, such as the direct benefits of fertility insurance, acquisition of nutrients, paternal care, avoidance of harassment, change of long-term partner or possible indirect benefits of genetic quality (see review Birkhead & Parker 1997). The relative role of these factors has not been established for the Grapsidae. However, it has been shown for spiny lobsters (Palinuridae), that female fertilisation success can be constrained by the supply of sperm (MacDiarmid & Butler 1999) and hence multiple matings or matings with large males are advantageous for females. Furthermore, female blue crab, *Callinectes sapidus*, receive significantly less sperm when they mate with a male that did not have enough time to recover seminal resources after a previous copulation (Kendall et al. 2002). Multiple matings could therefore be beneficial.

The grapsid males in this study quickly approached receptive females and mated with them. Male-male competition appeared to be the dominant factor in pair-formation in all four species, as large males were generally more successful competitors for females than medium and small males (Chapter 5, Fig. 5.9) and were more often the last males to mate (Table 8.1). No overt female choice based on male size was present as females usually mated with any male that approached and grasped them, although females occasionally struggled to resist advances by males, particularly in the case of *C. lavauxi*. However, females of another grapsid species, *Gaetice depressus*, appear to express some choice in preferentially approaching larger males for mating (Fukui 1994). Originally, I speculated that females could be 'choosy' by laying eggs after mating with a preferred male, if one assumes that the last male's sperm is used for fertilisation (see below). However, this was not apparent.

Males of *Hemigrapsus* spp. guarded females after mating until the females laid eggs, but not the other two species (Table 8.1) and this behaviour is currently not known in any other grapsid crab (Chapter 5, Table 5.2). In other species, postcopulatory guarding by males has been suggested to increases a male's reproductive success (Jivoff 1997a; see review Birkhead &

Møller 1998). However, females that are guarded by males are confronted with possible costs (e.g., restriction with regards to movement, unable to 'sample' other males, lost foraging time, risk of injury when a competitor attacks a pair, and possibly a higher predation rate for conspicuous pairs) and benefits (e.g., protection of vulnerable soft-shelled females in case of post-moult mating) of being guarded, and might therefore resist guarding attempts by males. Receptive female *C. lavauxi* and *H. crassa* were not guarded by males and could therefore move around and 'sample' other males. However, no evidence for female searching behaviour was found in the laboratory. Furthermore, post-copulatory guarding does not appear to determine the ultimate number of female matings because unguarded receptive female *C. lavauxi* mated less often and unguarded female *H. crassa* mated more often than guarded female *Hemigrapsus* spp. It appears that in Grapsidae mate guarding is primarily used by males to control access to receptive females.

Males of the two *Hemigrapsus* spp. followed a mating system in which they searched and defended mobile receptive females, of which few were available at any one time and place (female centered competition) (see also Chapter 5, Table 5.3). The relatively stable habitats of *H. sexdentatus* (i.e., mid-intertidal zone in relatively sheltered rocky, stony or muddy shores, where it typically hides under stones and boulders during low tide, often in small remaining puddles; McLay 1988) and *H. crenulatus* (i.e., mid- to low-intertidal zone in sheltered habitats, where it either hides under stones or buries in soft substrates such as mud and clay; McLay 1988) appear to give the crabs enough protection from desiccation, wave action, and predators to allow males to search for and defend receptive females for a prolonged period of time. Similarly, the search and defend mode of male competition is common in other aquatic Crustacea such as Portunidae and Cancridae which live in structurally complex environments and where females often produce eggs only once per lifetime or per reproductive season (Christy 1987). It was therefore argued that because females are rare and such an unpredictable resource in space and time and reproduce infrequently, a male's best strategy would be to search for and guard receptive females. Therefore, receptive females are encountered so rarely that they constitute a resource worth guarding.

In contrast, male *C. lavauxi* searched for and intercepted a receptive female only for the duration of mating and then moved on until they encountered another receptive female (encounter rate competition: pure search and interception). This strategy is likely to be an adaptation to the high shore habitat that *C. lavauxi* occupies. *Cyclograpsus lavauxi* lives among rocks and boulders and is frequently exposed to strong wave action during high tide and to air for several hours during low tide. *Cyclograpsus lavauxi* hides under rocks and boulders and holds

onto stones with the last pair of legs. The crab backs up diagonally against a stone with the last legs in sub-dorsal position and the other legs holding the crab off the substrate (McLay 1988). This behaviour is thought to stabilise the crab against the effect of the waves and to aid in mobility. In this habitat, perhaps males cannot easily stabilise themselves and guard receptive females at the same time for a prolonged period as they will get washed away by the water during high tide. In addition, females also carry the risk of getting washed away and therefore are likely to resist prolonged guarding attempts by males. Furthermore, the probability of desiccation (during low tide) and predation will be smaller for a single crab that is concealed and protected closely under a rock than a 'bulky' pair trying to fit underneath a stone.

Male *Helice crassa* searched for receptive females in their immediate neighbourhood and mated with them briefly on the substrate or in the burrow after which the female left (encounter rate competition: neighbourhoods of dominance) (Table 5.3). The high predation rates on the open mud flats coupled with the short lifetime of burrows (about one day; Sivaguru 2000), which are not used for egg incubation by the females, are likely to be important factors in the evolution of this male mating mode, in which males neither directly compete for females nor for resources.

Therefore, the different habitats and associated behaviour of finding physical protection from wave action and reducing the risk of desiccation and predation may play an important function in shaping the different mating system of *Hemigrapsus sexdentatus*, *H. crenulatus*, *C. lavauxi*, and *Helice crassa*, even though the spatial and temporal distribution of receptive females were similar in these species (see below).

A strongly skewed operational sex ratio (OSR) is expected when females have an asynchronous and brief period of sexual receptivity coupled with a long period of sexual receptivity for males (Emlen & Oring 1977). *Helice crassa* has an asynchronous mating season and a relatively short female receptive period which result in a highly male-biased OSR (Table 8.1). However, although *C. lavauxi* and *Hemigrapsus sexdentatus* had a synchronous mating season, their OSR was similarly strongly male-biased compared to *Helice crassa* because females of *C. lavauxi* and *Hemigrapsus crenulatus* were receptive for a shorter period than *Helice crassa* and because of differences in the general sex ratio (Table 8.1).

Males of the two *Hemigrapsus* species appeared to follow a different strategy of sperm allocation. Male *H. crenulatus*, which are typically confronted with a high mating frequency of the female and a long, asynchronous mating season, distribute similar-sized ejaculates, irrespective of female size. By contrast, male *H. sexdentatus*, which experience a comparatively lower risk of sperm competition during a short, synchronised mating season, invest larger ejaculates for larger females than for smaller females. Similarly, male *Panulirus argus* vary the

amount of ejaculate positively with female size (MacDiarmid & Butler 1999). In addition, the size of the first and second ejaculates transferred to a female by a male *H. crenulatus* were not significantly different, whereas the first was larger than the second for *H. sexdentatus* (see Chapter 3). Although, I did not examine the relationship between ejaculate size and sperm number for *Hemigrapsus spp.*, it is likely that larger ejaculates contain more sperm compared to smaller ejaculates as has been shown for *Chionoecetes opilio* (Sainte-Marie & Lovrich 1994) and *Callinectes sapidus* (Jivoff 1997b). For both *Hemigrapsus* species, male size did not affect the ejaculate size, meaning that small and large males transferred similar-sized ejaculates. Therefore, there is no advantage in this regard for a female to mate with a larger male.

Spermathecae were relatively full after the second mating and their weight increased only slightly with subsequent matings in *C. lavauxi* and *Hemigrapsus* spp. This indicates that the number of sperm stored does not increase at the same rate with an increased number of matings (unless the composition of the ejaculate changes such that sperm is transferred in a more concentrated form). Therefore, subsequent males (i.e., after second mating) appear to be able to transfer only smaller amounts of sperm to female spermathecae. All females had ventral-type spermathecae. This type is likely to promote last-male sperm precedence as has been shown for the ocypodid crab *Scopimera globosa* (Koga et al.1993) and some spider crabs, *Inachus phalangium* (Diesel 1989, 1991) and *Chionoecetes opilio* (Urbani et al. 1998). However, this remains to be shown for Grapsidae because recent studies on grapsid spermathecae (see Anilkumar et al. 1999; López Greco et al. 1999) did not provide conclusive evidence for the presence or absence of last-male sperm precedence.

After establishing the general mating behaviour of the four species, I investigated possible factors influencing female receptivity and the number of female matings (i.e., the extent of sperm competition) such as the absence or presence of males, competition between males, female size, and compared the behaviour in the laboratory and field.

Female receptivity

The absence or presence of males significantly affected the duration of female receptivity in the laboratory (Table 8.2) and field (Chapter 2). Isolated females stayed receptive significantly longer than females housed with males. This suggests that females have some control over the duration of their receptivity and therefore the time available for mating. For example, if males are not immediately available when a female becomes receptive, the female could extend the receptive period to increase the probability of acquiring a mate. The experimental modification

of the operational sex ratio had no effect on the duration of female receptivity (*Hemigrapsus crenulatus*, Chapter 4).

The duration of female receptivity was usually not affected by the size of females. However, a positive correlation between the duration of receptivity and female size was found for isolated *Hemigrapsus crenulatus* and *H. sexdentatus* temporarily housed with males (Table 8.2).

The onset of the mating season in the laboratory was influenced by the time females spent in the laboratory. The longer females were housed in the laboratory relative to the onset of the mating season in the field, the earlier they became receptive (Chapters 2 and 4). It has been shown previously that laboratory conditions can have an effect on crustacean behaviour (Williams 1969) and reproductive events (Little 1968; Sulkin et al. 1976; Zimmerman & Felder 1991). Similarly, it is likely that factors such as temperature and photo-period influenced the onset of the mating season in the laboratory. In addition, it appeared that the duration of receptivity was longer in the laboratory than in the field because comparatively few receptive females were found in the field and there was no indication that receptive females move out of the sampled area (Chapters 2 and 5). Therefore, observations made in the laboratory regarding female receptivity and mating season did not precisely indicate when the mating season in the field started and how long female remain receptive. Laboratory observations suggested that the breeding season started much earlier and that females remained receptive for a longer period. These results suggest that whenever possible, these important aspects of mating strategy should be also studied in the field.

Female matings, male-male competition and sperm competition

The number of times females mated was influenced by the temporary and constant presence of males. Females which were housed constantly together with males mated significantly more often than females which were housed only temporarily with males, except for *Hemigrapsus crenulatus* (Table 8.2). For *H. crenulatus*, the number of matings of females constantly together with males varied depending on the sex ratio, and was then either significantly higher or similar to females housed temporarily with males. When several males were constantly competing for one or two females (i.e., female to male OSR of 1:3 and 2:2), females mated almost twice as often as when only one male was present (i.e., female to male OSR of 2:1 and 1:1) (Chapter 4). Therefore, in *H. crenulatus* a more male-biased OSR, male-male competition increased the number of matings per female and hence sperm competition within the female spermathecae.

Similarly, male *C. sapidus* passed larger ejaculates in the presence of rivals presumably as a response to sperm competition (Jivoff 1997a).

The number of matings per female was in some cases correlated with the receptive duration but not in others (Table 8.2). For example, female matings increased with the receptive duration when females were housed temporarily or constantly with males for *Hemigrapsus crenulatus* and when females were temporarily together with males in *H. sexdentatus*, but no such correlation was found for *C. lavauxi* and *Helice crassa*. The number of female matings was not affected by female size.

In the laboratory, where crabs were constantly submerged under water, the time of day (day *vs.* night) did not affect the mating frequency for any of the four species investigated. However, in the field, a lunar or tidal cycle may influence the time of mating as has been suggested for other grapsid crabs (Zimmerman & Felder 1991) and shown for several ocypodid crabs (Henmi & Murai 1999; Murai et al. 2002). In the field, pairs of *C. lavauxi* and *H. sexdentatus* were found in copula underneath rocks, and pairs of *Helice crassa* were observed mating on the substrate as well as in burrows, during low tide. It is not known whether crabs also mate during high tide in the field, as this was not investigated.

It was estimated from the weight of female spermathecae that in the field female *C. lavauxi* mate at least once and possibly more often, female *H. crenulatus* between two and six times, and female *H. sexdentatus* up to two times. The number of female *C. lavauxi* matings in the laboratory (Table 8.1) was therefore in the range of estimated field matings. However, the two *Hemigrapsus* spp. appear to mate less often in the field than in the long-term trials in the laboratory (Table 8.1). A shorter receptive period of females in the field could explain these differences. In addition, the densities of crabs during the experiments in the laboratory were probably higher compared to the field and receptive females were immediately exposed to males which made it easy for the males to locate receptive females.

The above findings have several implications for our current understanding of mating strategies in Grapsidae, which are more diverse than previously thought (see Chapter 5). The mating system appeared to be an adaptation to the habitat crabs occupy and the temporal and spatial distribution of receptive females. Furthermore, females with a restricted duration of sexual receptivity seem to have some control over their receptive period and can therefore influence the OSR and the extent of male-male competition. Increased male-male competition in turn is expected to ultimately benefit a female's reproductive success. As females mated multiple times during their receptive period, sperm competition is a common feature in

Grapsidae. However, males employed different tactics in regards to sperm competition such as longer mating duration (e.g., *C. lavauxi*), high number of matings (*Helice crassa*), or post-copulatory mate guarding until oviposition (*Hemigrapsus* spp.).

Parasite fauna of four grapsid crabs: host-parasite associations and effects on reproduction

This study documented four internal parasite species (Table 8.3), of which two were described for the first time (Brockerhoff & Smales 2002 (Appendix 10.1); Poinar & Brockerhoff 2001 (Appendix 10.2)). Previously, none of the investigated grapsid crabs was known to be infected by any parasite. Each crab species harboured at least one parasite species but could host up to three (Table 8.3). The internal parasites found in the crabs came from a wide taxonomic range, i.e., Crustacea, Acanthocephala, and Nematomorpha. The effects of the parasites on their hosts were shown to vary depending on the parasite's biology. In general, the effects of parasites on host phenotypes can include changes in motor activity (increased or decreased activity), behaviour (e.g., altered microhabitat choice), or morphology (e.g., altered coloration or body size) (Poulin & Thomas 1999). Furthermore, mating behaviour can be affected by a range of parasites such as nematodes and nematomorphs (Moore & Gotelli 1990).

The entoniscid isopod *Portunion* sp. (Chapter 6) has a complex life cycle which probably includes a copepod intermediate host and a crab final host. Therefore, after infecting the crab, no transmission to another host for reproduction of the parasite is necessary and therefore there is no necessity for the parasite to influence the host behaviour to increase its transmission to the next host. However, the adult parasite might still influence crab behaviour when it releases its larvae to enhance the transmission of larvae to the copepod intermediate host. I showed that *Portunion* sp. differentially affects male and female grapsid hosts. Females were castrated whereas the internal organs of males did not show any morpholgical changes. Furthermore, the ability of infected males to mate was not different from uninfected males and infected males were equally successful in competing with uninfected males for females. Thus, *Portunion* sp. altered the operational sex ratio causing it to be more male-biased and thereby influenced the intensity of male-male competition and, consequently, sexual selection. Furthermore, due to the sometimes high prevalence of *Portunion* sp. in the host population it has the potential to substantially affect host population dynamics such as reduced reproduction and recruitment.

The acanthocephalans *Profilicollis* spp. (Polymorphidae) have a complex life cycle in which the crabs are used as intermediate hosts and vertebrates as definitive hosts.

Acanthocephalan cystacanths are transmitted by predation. Although not investigated here, it is likely that *Profilicollis* causes some behavioural changes to the crabs to enhance the probability of successful transmission to the final host (birds) as has been shown for other Acanthocephala (Moore 1984). An increase of the activity level and an altered behavioural response often caused by Acanthocephala can lead to higher predation rates of infected hosts compared to uninfected hosts (see review Moore & Gotelli 1990). In these cases the mortality of infected hosts is enhanced and this can affect host population dynamics.

The nematomorph *Nectonema zealandica* (Nectonematoidea) presumably has a relatively simple life cycle similar to other species in the genus in which juveniles utilise crustaceans as hosts whereas the adult aquatic worms are free living (Overstreet 1983). Therefore, *Nectonema* does not need to change its host behaviour to enhance transmission to another host. However, the nematomorph larva could influence the host's behaviour to be released at a favourable location as has been suggested for some species of the order Gordioidea (Poinar 1991). The impact of *N. zealandica* on crab mating behaviour was not investigated in this study because of the lack of parasitised crabs (harbouring live parasites, see Appendix 10.2) used in the experiments. However, it has been shown that female grasshoppers (*Orchelimum gladiator*) infected by a nematomorph from the order Gordioidea were less likely to mate or respond to mating stimuli (Morris et al. 1975). In addition, castration can occur in female shrimps, *Palaemonetes vulgaris*, infected with *Nectonema agile* (Born 1967), and *Nectonema* sp. may be responsible for atrophied gonads in male rock crabs, *Cancer irroratus* (Leslie et al. 1981). In contrast, other host species such as the crabs *Cancer borealis*, *Pagurus acadianus*, *Munida tenuimana* and the shrimps *Pandalus montagui* and *P. borealis* were not castrated (Nielsen 1969; Leslie et al. 1981). Similarly, no pathological effect was apparent in the present study in *Hemigrapsus sexdentatus* infected by *N. zealandica*. Furthermore, the high rate of dead larvae of *N. zealandica* found in *H. sexdentatus* suggests that this crab species is not the preferred host, since in long-term parasitic associations, lethal host reactions are rare. Marine nematomorphs are known not to be host-specific (see Appendix 10.2) and although a survey of other infected marine crustaceans in the Waipara area has not been undertaken, many other potential hosts are likely to be present.

The prevalence of the parasites was studied in relation to host size and gender (Table 8.4). A significant positive correlation between parasite prevalence and host size was found in the case of *Portunion* sp., but not for *Profilicollis* spp. and *N. zealandica*. In general, the relationship between parasite prevalence and host size can potentially be the result of a number

of factors such as differential growth and survivorship of parasitised hosts or differential probability of infection of different size classes (O'Brian and van Wyk 1985). *Portunion* sp. is likely to accumulate over time in the larger, and therefore older, crabs because older crabs have been exposed longer to potential infection. Once the crab is infected, it appears that *Portunion* sp. grows and reproduces until the crab dies, probably with only limited parasite mortality in the meantime as has been shown for *P. conformis* (Kuris et al. 1980). In contrast, in the case of *Profilicollis* spp. parasite prevalence is likely to be partially controlled by the selective removal of infected crabs by the definitive hosts inhibiting an accumulation in larger size classes (see discussion Chapter 7). Therefore, birds feeding selectively on parasitised crabs and possibly on a particular size group have the potential to affect parasite prevalence in relation to host size. The extent of this relationship has yet to be established as other biotic and abiotic factors, such as the possibility of an increased defence capability of older crabs, have yet to be investigated. *Nectonema zealandica* utilises the crabs only temporarily for its larval development and then leaves its host. Therefore, it is not surprising that the prevalence of *N. zealandica* does not correlate with host size unless a particular size class was more likely to be parasitised.

Parasite prevalence in relation to host gender varied among the parasite species investigated such that either male or female hosts had a higher prevalence or no differences between the sexes were found (Table 8.4). It is not known why differences between the prevalence of males and females occur, particularly as male and female crabs inhabit similar habitats and are likely to have similar feeding habits. One would therefore expect that they are exposed to the same number of infective stages. One possible explanation could be differences in behaviour associated with carrying eggs. For example, ovigerous females could temporarily choose a different microhabitat compared to that of males, e.g., spending more time hiding under rocks or in burrows, or females engage in more grooming activity which could change the probability of infection. Shields (1992) suggested that a greater parasite diversity and abundance occurs in mature female than in mature male *Portunus pelagicus* because females may have a slower growth rate and hence acquire more fouling organisms. In addition, female *P. pelagicus* may have different feeding and migratory habits than males which may have caused a higher cestode load and the female egg clutch provided a unique microhabitat for egg predators.

It has been discussed that a higher susceptibility to diseases or parasites in males than in females might be a consequence of the different reproductive strategies favoured by sexual selection (Sheridian et al. 2000). In vertebrates, for example, it has been argued that the immunosuppressant effects of testosterone cause higher parasite infections in males. In comparison arthropods do not display a clear trend for differences in male and female prevalence

of parasite infections possibly because of the absence of endocrine-immune interaction in arthropods (Sheridian et al. 2000). However, if significant differences in male and female prevalence in arthropods occur they might be explainable by the differences in habitat or behaviour of males and females.

In summary, this study described several parasites from grapsid hosts for the first time and two of them were new to science. The parasites found differentially affected their crab hosts. The parasitic castrator *Portunion* sp. suppressed reproduction of the female hosts and caused a male-biased shift of the operational sex ratio. The acanthocephalans *Profilicollis* spp. were likely to influence crab behaviour to enhance their transmission to the next host and thereby cause an increased mortality of infected hosts. No effect of the nematomorph *N. zealandica* was apparent. However, this study provided mainly an overview on parasite prevalence and parasite occurrence in relation to host gender and size, and more experimental work is necessary to elucidate the full impact of the parasites on host reproduction and survival.

Table 8.1 Receptivity and mating behaviour of female *Cyclograpsus lavauxi* (n = 13), *Helice crassa* (n = 9), *Hemigrapsus crenulatus* (n = 11), and *Hemigrapsus sexdentatus* (n = 14) in long-term laboratory trials until oviposition (sex ratio: 1 female to 3 males (large, medium, small)) using continuous video recording, and the duration of the mating season and the operational sex ratio (OSR, receptive female per males) in the field.

Species	Duration of female receptivity until oviposition (d)[1]	Female matings per receptive period prior to oviposition [1]	Duration of copulation (min) [1]	Prolonged post-copulatory guarding until oviposition	Last male to mate (large – medium – small males)	Mating after oviposition	Duration of calcification of gonopore opercula after oviposition	Mating seasons in the field, estimated duration	OSR in the field and males per receptive female in parenthesis
Cyclograpsus lavauxi	6.3 ± 0.7 (2 - 10)	2.1 ± 0.2 (1 - 3)	116.1 ± 11.1 (11 - 251)	no	53.8% – 30.8% – 15.4%	no	1 d	one synchronous, about 4 weeks	0.010 – 0.053 (19 – 99 males)
Helice crassa	12.4 ± 0.7 (10 - 15)	24.3 ± 4.6 (5 - 51)	14.6 ± 0.5 (4 - 76)	no	66.7% – 22.2% – 11.1%	common	2 - 3 wk	at least two asynchronous, both within about 6 months	OSR*: 0.063 – 0.309 (3 – 16 males) OSR**: 0.015 – 0.077 (13 – 67 males)
Hemigrapsus crenulatus	4.1 ± 0.7 (1 - 8)	7.5 ± 1.6 (2 - 15)	15.7 ± 0.6 (4 - 35)	yes	72.7% – 27.3% – 0%	rarely	1 - 2 d	two asynchronous, both within about 6 months	-
Hemigrapsus sexdentatus	5.5 ± 0.4 (3 - 9)	7.8 ± 0.8 (2 - 13)	9.4 ± 0.5 (3 - 27)	yes	64.3% – 14.3% – 21.4%	no	2 - 4 d	one synchronous, about 3 weeks	0.027 – 0.074 (14 – 37 males)

1, the mean with standard error is followed by the range in parenthesis; OSR*, all receptive females (with and without eggs); OSR**, receptive females without eggs.

Table 8.2 Female receptivity, mating frequency, and female size, as factors in female reproductive behaviour of four grapsid crabs. Observations were carried out for isolated females (isol., fem_{isol}) and females housed temporarily (temp., fem_{temp}) or constantly (const., fem_{const}) with males during their receptive period in the laboratory. Fem_{temp} were together with two males (large and small male) and fem_{const} were together with three males (large, medium, and small male) unless otherwise stated (see *Hemigrapsus crenulatus*).

Species / experiments carried out	Comparison of duration of receptivity	Comparison of number of matings	Receptivity *vs.* no. of matings	Receptivity *vs.* female size
Cyclograpsus lavauxi temp. & const.	$fem_{temp} = fem_{const}$	fem_{const} *> fem_{temp}	no correlation for fem_{temp} and fem_{const}	no correlation for fem_{temp} and fem_{const}
Helice crassa const.	-	-	no correlation fem_{const}	no correlation for fem_{const}
Hemigrapsus crenulatus isol., temp., & const.	fem_{isol} *> fem_{temp} *> fem_{const}	fem_{const} (OSR, 2:2, 1:3) *> fem_{temp}, fem_{const} (OSR, 2:1, 1:1) = fem_{temp}	*pos. correlation for fem_{temp} and fem $_{const}$	no correlation for fem_{temp} and fem_{const}, *pos. correlation for fem_{isol}
Hemigrapsus sexdentatus isol., temp., & const.	fem_{isol} *> fem_{temp} & fem_{const} combined	fem_{const} *> fem_{temp}	*pos. correlation for fem_{temp}, no correlation for fem_{const}	no correlation fem_{isol} and fem_{const}, *pos. correlation for fem_{temp}

*, statistically significant differences; OSR, operational sex ratio presented as the actual no. of crabs used in the experiments.

Table 8.3 Prevalence of internal parasites found in four grapsid crabs collected from three sites in Canterbury, New Zealand.

Host species / collection site	**Parasite species**		
	***Portunion* sp.** (Crustacea)	***Profilicollis novaezelandensis* & *Profilicollis antarcticus*** (Acanthocephala)(combined)	***Nectonema zealandica*** (Nematomorpha)
Cyclograpsus lavauxi Lyttelton Harbour	34.1%	0%	0%
Helice crassa Avon-Heathcote Estuary	11.6%	4.1%	0%
Hemigrapsus crenulatus Lyttelton Harbour	19.0%	26.5%	0%
Hemigrapsus sexdentatus Beach near Waipara	0%	0%	12.7%

Table 8.4 Prevalence of internal parasites found in four grapsid crabs related to host gender and size.

Parasite species	**Parasite prevalence**	
	correlation with host size	**host gender**
***Portunion* sp.** (Crustacea)	positive*	males > females[1,]* males = females[2]
***Profilicollis* spp.** (Acanthocephala)	no	males = females
Nectonema zealandica (Nematomorpha)	no	females > males*

1, in *Cyclograpsus lavauxi* and *Hemigrapsus crenulatus*; 2, in *Helice crassa*; *, statistically significant

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Profilicollis novaezelandensis n. sp. (Polymorphidae) and two other acanthocephalan parasites from shore birds (Haematopodidae and Scolopacidae) in New Zealand, with records of two species in intertidal crabs (Decapoda: Grapsidae and Ocypodidae)

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Abstract

Profilicollis novaezelandensis n. sp. (Acanthocephala: Polymorphidae) is described from the South Island pied oystercatcher *Haematopus ostralegus finschi* Martens (Haematopodidae) and the intertidal crab *Hemigrapsus crenulatus* (Milne Edwards) (Brachyura: Grapsidae) from the South Island of New Zealand. The new species can be distinguished from all the other species of the genus by a combination of the following characters: long neck (13% of total body length for adults) and a subspherical proboscis with 14–16 longitudinal rows of 7–8 hooks. The mud crabs *Helice crassa* Dana (Grapsidae) and *Macrophthalmus hirtipes* (Heller) (Ocypodidae) were also harbouring cystacanths and the bar-tailed godwit *Limosa lapponica* (Linnaeus) (Scolopacidae) juveniles of *P. novaezelandensis*. This is the first record of brachyuran decapods as intermediate hosts of Acanthocephala from New Zealand. *P. antarcticus* is recorded from three crab species (*Helice crassa*, *Hemigraspus crenulatus* and *Macrophthalmus hirtipes*) and two bird species (*Haematopus o. finschi* and *Limosa lapponica*) in New Zealand. An unidentified species of *Plagiorhynchus* was also found in two bird species (*H. o. finschi* and *H. unicolor* Forster). *P. antarcticus* and *P. novaezelandensis* are the first records of *Profilicollis* from New Zealand.

Introduction

The Polymorphidae Meyer, 1931 (Acanthocephala) is a homogeneous assemblage of species comprising 10 genera (Amin, 1992; Dimitrova & Georgiev, 1994; Nickol, Crompton & Searle, 1999) most of which are parasites of aquatic birds and mammals (Schmidt, 1973). The life-cycles typically include a crustacean intermediate host and may include a fish paratenic host (Schmidt, 1985). *Profilicollis* Meyer, 1931 is characterised, amongst other traits, by using a decapod crustacean as the intermediate host (Nickol et al., 1999).

South Island pied oystercatchers *Haematopus ostralegus finschi* Martens usually feed in large flocks in estuaries and mudflats where bivalves are abundant but their diet also includes a variety of marine invertebrates including small mud crabs like *Helice crassa* Dana (Grapsidae) (Baker, 1974). Variable oystercatchers *Haematopus unicolor* Forster are usually sparsely distributed over rocky shores from which they take mainly limpets, chitons and mussels, but also include crabs such as *Helice crassa* and *Hemigrapsus edwardsii* Hilgendorf in their diet (Baker, 1974). Bar-tailed godwits *Limosa lapponica* (Linnaeus) are summer visitors to New Zealand shores (Higgins & Davies, 1996). It is possible that they also take intertidal crabs such as *Hemigrapsus* as has been reported for *Limosa fedoa* Linnaeus (see Gratto-Trevor, 2000).

Currently, there is a general lack of data on the acanthocephalan parasites of shore birds and marine invertebrates in New Zealand, with no comprehensive survey having been carried out to date. Only *Plagiorhynchus* sp. from the South Island pied oystercatcher *H. o. finschi*, *Polymorphus* sp. from the pied stilt *Himantopus himantopus* Linnaeus (see McKenna,

1998), *Corynosoma* sp. from the gentoo penguin *Pygoscelis papua* Forster, *Corynosoma clavatum* Goss, 1941 from the Auckland Island shag *Leucocarbo campbelli colensoi* Buller and *Echinorhynchus* sp. from the North Island brown kiwi *Apteryx australis mantelli* Bartlett (see Weekes, 1982) have been recorded. The aims of the present study were therefore to examine three species of shore birds and five species of intertidal crabs for stages in the life-cycle of acanthocephalan parasites. Two species of *Profilicollis* were found, one of which is a new species and is described below. A *Plagiorhynchus* species, possibly that of McKenna (1998), was also found, determined to be new and will be described elsewhere.

Materials and methods

A total of 206 *Haematopus ostralegus finschi* (South Island pied oystercatchers) were collected between 1971 and 1980 from four sites: the Avon-Heathcote Estuary (43°33′S, 172°44′E); the mouth of the Ashley River (43°17′S, 172°43′E); Parapara, Golden Bay (40°43′S, 172°42′E) on the east coast of the South Island; and Anaura Bay (38°14′S, 178°20′E) on the east coast of the North Island. Five *H. unicolor* (variable oystercatchers) were collected at the mouth of the Moeraki River (43°42′S, 169°15′E) and Gillespie Beach (43°25′S, 169°49′E) on the west coast of the South Island in 1971. Seventeen *Limosa lapponica* (bar-tailed godwits) were collected from the Avon-Heathcote Estuary and the mouth of Ashley River between 1973 and 1980.

Five intertidal crab species were collected from three sites in 1998 and 1999: a total of 1,970 *Hemigrapsus crenulatus* (Milne Edwards), 1,650 *Cyclograpsus lavauxi* (Milne Edwards) (Grapsidae) and 225 *Macrophthalmus hirtipes* (Heller) (Ocypodidae) were collected from Governors Bay in Lyttelton Harbour (43°38′S, 172°39′E), 827 *Helice crassa* (Grapsidae) from the Avon-Heathcote Estuary, and 448 *Hemigrapsus edwardsii* Hilgendorf (Grapsidae) from a beach near Waipara (43°06′S, 172°53′E) from the east coast of the South Island.

Adult acanthocephalans were fixed in 10% formalin or alcohol-formalin-acetic acid (AFA) and stored in 70% ethanol prior to examination. Cystacanths were placed in tap-water until the proboscis everted, then fixed in AFA or 70% ethanol. Specimens examined under light microscopy were dehydrated in 96% ethanol and cleared in beechwood creosote. Cystacanths selected for SEM were fixed in 2.5% glutaraldehyde, buffered with 0.1 M Na cacodylate (pH 7.3), followed by a buffer wash, post-fixation in 1% buffered osmium tetroxide, a buffer wash, a graded ethanol dehydration series into 100% ethanol and a graded transfer into 100% amyl acetate. Specimens were dried to critical point using liquid CO_2, mounted onto aluminium stubs using conductive carbon paint and sputter coated with 50 nm of gold/palladium in a vacuum desiccator. They were viewed with a Leica S440 SEM at accelerating voltages of 15–20 kV.

Drawings and measurements were made with the aid of a camera lucida and are given in micrometres as the range, followed by the mean in parentheses, unless otherwise stated. Eggs were measured through the body wall. Width measurements refer to maximum width. Specimens have been deposited in the National Museum, Wellington, New Zealand (NMNZ).

Results

Parameters of infestation

Of the 206 pied oystercatchers surveyed 2.2% from the Avon-Heathcote Estuary, 8.5% from the Ashley River, 30.0% from Parapara and one of three from Anaura Bay harboured Acanthocephala. Two of five variable oystercatchers from Gillespie Beach and Moeraki and nine of 17 bar-tailed godwits from the Avon-Heathcote Estuary and Ashley River were also found to be infected with acanthocephalans. Three species were identified, *Plagiorhynchus* (*Plagiorhynchus*) sp., which is being described elsewhere, and two species of *Profilicollis* (Table 1). Cystacanths of *Profilicollis* spp. (see descriptions below) were found in 26.5% of *Hemigraspus crenulatus*, in 4.1% of *Helice crassa* and 19.5% of *Macrophthalmus hirtipes*. No cystacanths were found in *Hemigrapsus edwardsii* and *Cyclograpsus lavauxi*.

Profilicollis antarcticus Zdzitowiecki, 1985

The most common acanthocephalan, occuring in 2 bird hosts, was determined to be *Profilicollis antarcticus* Zdzitowiecki, 1985, following the key of Amin (1992).

Material examined: Adults from the small intestine of *Haematopus ostralegus finschi* and *Limosa lapponica* collected from the Avon-Heathcote Estuary,

Table 1. Occurrence of Acanthocephala in oystercatchers and godwits from New Zealand.

Host species and locality	Date of collection	No. of birds examined/ infected	Acanthocephala prevalence (%) and parasite samples examined (*n*)	Parasites found [4]		
				Profilicollis novaezelandensis n. sp.	*Profilicollis antarcticus*	*Plagiorhynchus* sp.
Haematopus ostralegus finschi (S. I. pied oystercatcher)						
Anaura Bay (Gisborne)[2]						
	Jul. 1973	2 / 1	50% (1)		+	
	Aug. 1973	1 / 0	0%			
	1973	**3 / 1**	**33%**			
Ashley River[1]						
	Mar. 1980	12 / 1	8% (0)			
	Apr. 1980	2 / 0	0%			
	May. 1976	25 / 3	12% (3)	+	+	+
	Jul. 1973	8 / 0	0%			
	1973–1980	**47 / 4**	**9%**			
Avon-Heathcote Estuary[1]						
	Feb. 1971	17 / 0	0%			
	Apr. 1971	37 / 1	3% (0)			
	Apr. 1980	4 / 0	0%			
	Jun. 1971	15 / 0	0%			
	Jul. 1970	17 / 1	6% (0)			
	Sep. 1970	2 / 0	0%			
	Sep. 1976	7 / 0	0%			
	Oct. 1973	18 / 0	0%			
	Nov. 1979	3 / 0	0%			
	Dec. 1979	16 / 1	6% (1)			+
	1971–1980	**136 / 3**	**2%**			
Parapara (Golden Bay)[1]						
	Apr. 1971	**20 / 6**	**30%** (3)		+	
Haematopus unicolor (variable oystercatcher)						
Gillespie Beach[3]						
	Aug. 1971	3 / 1	33% (1)			+
Moeraki[3]						
	Jun. 1971	2 / 1	50% (1)			+
	1971	**5 / 2**	**40%**			
Limosa lapponica (bar-tailed godwit)						
Ashley River[1]						
	Mar. 1980	1 / 0	0%			
	Dec. 1979	2 / 1	50% (0)			
	1979–1980	**3 / 1**	**33%**			
Avon-Heathcote Estuary[1]						
	Apr. 1980	2 / 2	100% (1)		+	
	Oct. 1973	2 / 2	100% (2)		+	
	Dec. 1979	10 / 4	40% (4)	+	+	
	1973–1980	**14 / 8**	**57%**			

[1] East coast, South Island;
[2] East coast, North Island;
[3] West coast, South Island;
[4] Identified parasites are marked '+'.

Parapara, and Ashley River from the South Island, and Anaura Bay from the North Island, New Zealand, between 1971 and 1980, by B. Allison. Voucher specimens NMNZ ZW1483, ZW1484, ZW 1485, ZW1486. Cystacanths from the posterior haemocoel of *Helice crassa*, *Hemigrapsus crenulatus* and *Macrophthalmus hirtipes* collected from Governors Bay and the Avon-Heathcote Estuary by A. Brockerhoff 1998, 1999. Voucher specimens found in *H. crassa*, NMNZ ZW 1497.

Description

Adult

With large spherical proboscis with armature of 20–22 rows of 8 hooks; hook VI largest, *c.* 67; males with 4 cement-glands. Congruent with description of *P. antarcticus* by Zdzitowiecki (1985).

Cystacanth (Figures 1–4)

Based on 10 specimens collected from *Helice crassa*. White to orange. Females larger than males. Trunk bipartite; anterior part cylindrical, thin-walled, armed with numerous small spines directed posteriorly; posterior part ovoid, thick-walled, unarmed. Proboscis ovoid, armed with 22 rows of 6-8 hooks (usually 7). Neck long, 2/3 length of anterior part of trunk. Proboscis receptacle extends 2/3 into anterior trunk. Lemnisci highly variable in shape and size, often coiled in anterior trunk or extending into posterior trunk.

Male (n = 5). Total length of everted cystacanth 3,055–3,796 (3,390). Proboscis 398–455 (425) long, 316–347 (333) wide. Proboscis hooks: I 30–50 (44), II 42–61 (52), III 42–61 (53), IV 35–53 (48), V 38–64 (54), VI 50–66 (59), VII 48–67(62), VIII 45–66 (56). Proboscis receptacle 1,495–1,639 (1,548) long, 225–269 (242) wide. Neck 716–955 (827) long, 291–448 (358) wide. Anterior trunk 827–1,193 (981) long, 537–587 (564) wide; posterior trunk 1,114–1,193 (1,156) long, 568–726 (640). Somatic spines 15–17 (16) long, 8–10 (9) wide. Lemnisci, curled up, 907 long, (n = 1). Testes 2, ovoid, either both in anterior or posterior trunk or one in each part; anterior testis 138–141 (139) long, 102–117 (110) wide; posterior testis 131–157 (144) long, 90–120 (106) wide. Cement-glands not observed.

Female (n = 5). Total length of everted cystacanth 3,751–4,530 (4,181). Proboscis 505–537 (522) long, 392–417 (408) wide. Proboscis hooks: I 32–46 (39), II 51–66 (56), III 53–66 (58), IV 40–48 (44), V 50–61 (56), VI 53–64 (60), VII 58–64 (61), VIII 48–64 (58). Proboscis receptacle 1,957–2,259 (2,062) long, 247–285 (266) wide. Neck 891–1,225 (1,088) long, 316–429 (373) wide. Anterior trunk 1,130–1,400 (1,260) long, 632–701 (648) wide; posterior trunk 1,225–1,368 (1,384) long, 682–764 (733) wide. Somatic spines 15–17 (16) long, 8–10 (9) wide. Lemnisci, curled up, 811–827 (819) long.

Remarks

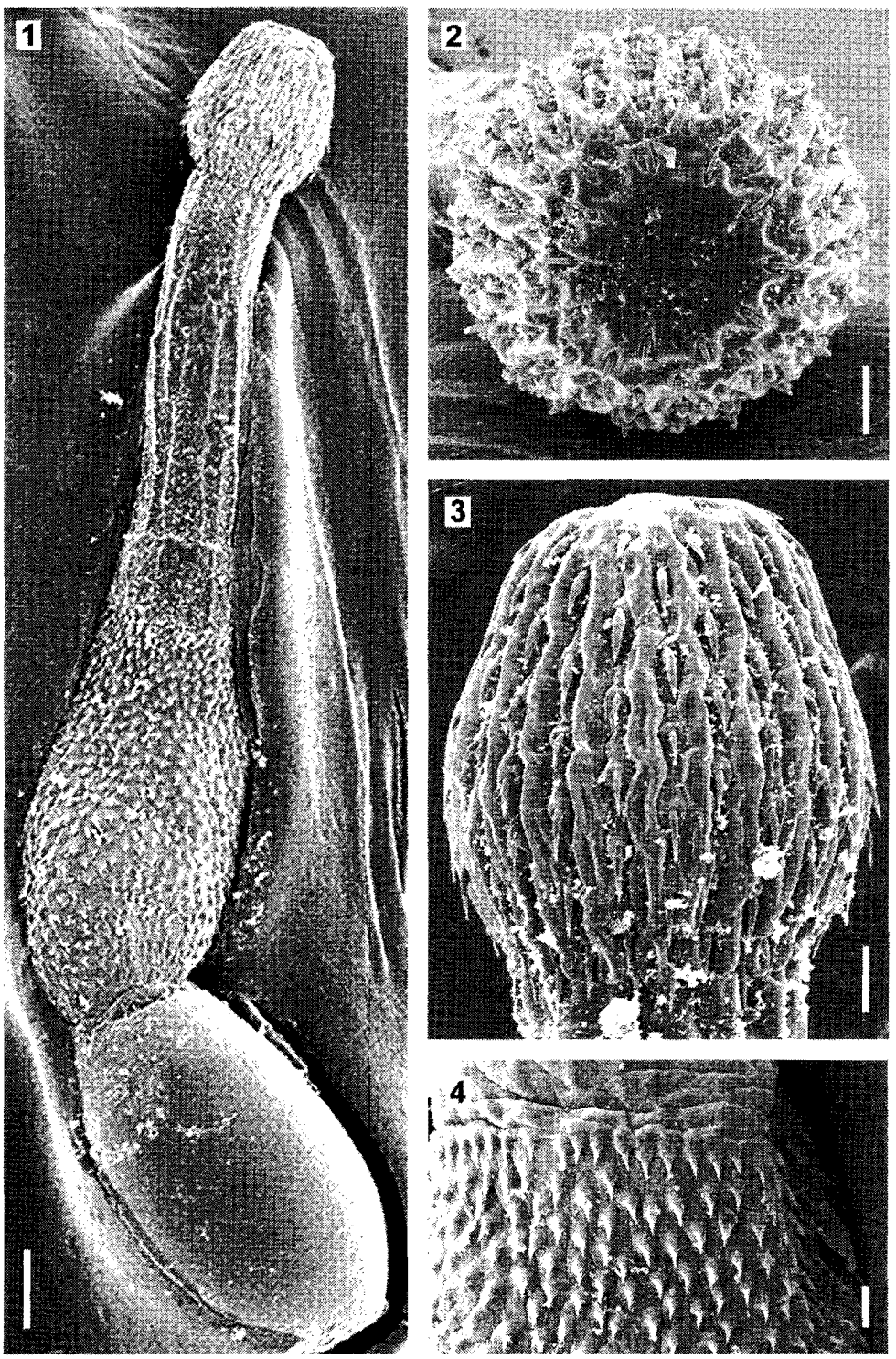
Profilicollis sphaerocephalus (Bremser in Rudolphi, 1819) is the only other species of *Profilicollis* previously recorded from the Southern Hemisphere (Zdzitowiecki, 1985). Although very similar to *P. antarcticus* (see Zdzitowiecki, 1985), *P. sphaerocephalus* has six cement-glands. Moreover, the eggs of *P. sphaerocephalus* are 60–66 ×30–37 (Johnston & Edmonds, 1947; Petrochenko, 1958), as compared with 73–93 × 26–30 in this study.

The proboscis armature and morphology of the cystacanth was consistent with either *P. antarcticus* (the cystacanths of which have not been previously described) or *P. sphaerocephalus* as described by Pichelin et al. (1998). Given that *P. antarcticus* was identified from the birds and not *P. sphaerocephalus*, these cystacanths are presumed to be *P. antarcticus*.

P. antarcticus is now recorded from the definitive hosts *Haematopus o. finschi*, *H. unicolor* and *Limosa lapponica* from New Zealand as well as *Chionis alba* (Gmelin) and *Larus dominicanus* Lichtenstein from the South Shetland Islands, Antarctica and Chile (Zdzitowiecki, 1985; Torres et al., 1991, 1992), and the intermediate hosts *Hemigrapsus crenulatus* from New Zealand (this study) and Chile (Pulgar et al., 1995), as well as *Macrophthalmus hirtipes* and *Helice crassa* from New Zealand (this study). All the New Zealand records are new locality records and except for *H. crenulatus* are also new host records.

***Profilicollis novaezelandensis* n. sp.**

Types: Holotype male, allotype female from the small intestine of *Haematopus ostralegus finschi* Martens, Ashley River, Canterbury, New Zealand, collector B. Allison, 20 May 1976; NMNZ holotype, allotype; paratypes 2 males, 4 females, 6 juveniles (2 males, 4 females) NMNZ 1487.



Figures 1–4. Cystacanth of *Profilicollis antarcticus* from *Helice crassa*. 1. Cystacanth, everted; 2. Proboscis, *en face* view, note clear distinction of 22 longitudinal rows of hooks; 3. Proboscis, lateral view; 4. Small spines on anterior trunk. *Scale-bars*: 1,200 μm; 2,3, 60 μm; 4, 40 μm.

Cystacanths: From the posterior haemocoel of *Hemigrapsus crenulatus* (Milne Edwards), Governors Bay in Lyttelton Harbour, New Zealand, collector A. Brockerhoff, 1998, 1999; NMNZ ZW 1496.

Other material: Two immature adults from the small intestine of *Limosa lapponica* (Linnaeus), Avon-Heathcote Estuary, Christchurch, New Zealand, coll B. Allison 7 Dec 1979; NMNZ 1488. Cystacanths from the posterior haemocoel *Helice crassa* Dana, Avon-Heathcote Estuary, Christchurch, and *Macrophthalmus hirtipes* (Heller), Governors Bay in Lyttelton Harbour, New Zealand, collector A. Brockerhoff, 1998, 1999.

Description (Figures 5–12)

Adult

Based on 3 males, 5 females (types). Trunk fusiform; male smaller than female. Proboscis subspherical, armed with 14–16 longitudinal rows of 7–8 hooks (usually 7); thorns with hooked tips; roots simple; hooks II–III largest, roots longer than thorns; posterior hooks becoming more slender, rootless; hooks I 70–95, II 80–110, III 85–105, IV–VI 75–80, VII 60–65. Neck long, conical, spineless, broader at base than anteriorly. Proboscis receptacle long, double-walled, attached to body wall at base of proboscis. Lemnisci long, flat. Anterior quarter of trunk covered by small spines, distributed equally on ventral and dorsal surfaces, extending to level of anterior testis in male.

Male. Trunk 4.4–14 mm long, 1.4–2.4 mm wide. Proboscis 450–646 (532) long, 402–475 (437) wide. Neck 952–1,190 (1,055) long, 442–555 (502) wide at base. Proboscis receptacle 1,785–2,210 (1,998) long, 204–340 (272) wide. Lemnisci extend posteriorly beyond level of proboscis receptacle, 2,300–3,100 long. Testes tandem; anterior testis 382–1,360 (915) long, 228–850 (530) wide; posterior testis 335–1,360 (885) long, 255–850 (590) wide. Four tubular cement-glands. Bursa not extended. Genital pore terminal; genital spines absent.

Female. Trunk 8–22 mm long, 1.8–2.5 (2.25) mm wide. Proboscis 510–646 (583) long, 435–595 (515) wide. Neck 804–1,190 (1,009) long, 400–630 (470) at base. Proboscis receptacle 850–1,785 (1,483) long, 338–340 (339) wide. Lemnisci and genitalia obscured by eggs. Eggs without polar prolongations, 49–59 (53.5) × 17–19 (17.3). Genital pore terminal; genital spines absent.

Cystacanth

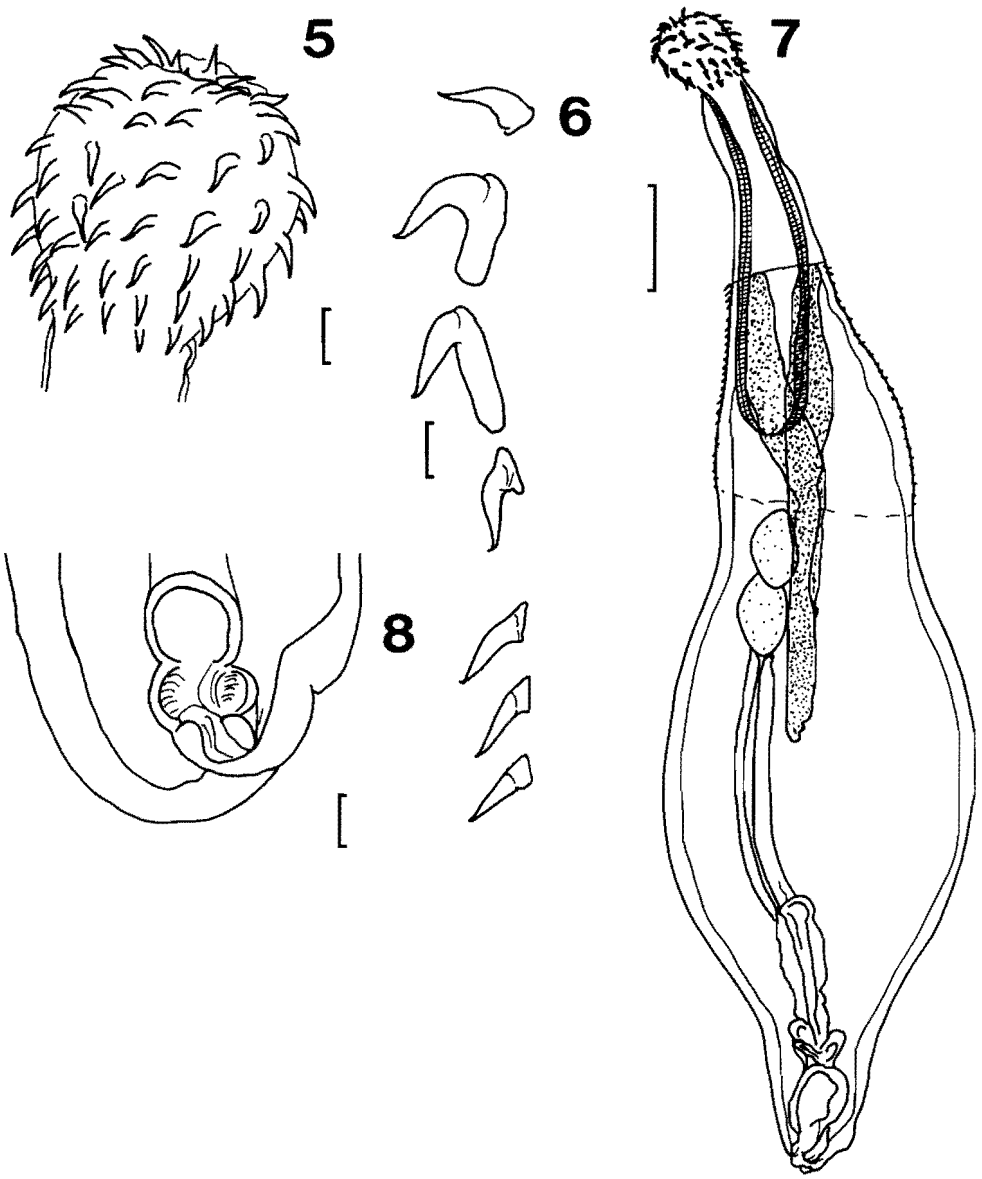
Based on 22 specimens collected from *Hemigrapsus crenulatus*. White to orange. Females larger than males. Trunk bipartite; anterior part cylindrical, thin-walled, armed with numerous small spines directed posteriorly; posterior part ovoid, thick-walled, unarmed. Proboscis ovoid, armed with 14 rows of 7–8 hooks (usually 7). Hooks I–III robust (curved posteriorly); hooks IV–VII more slender and straight. Neck long, 2/3 length of anterior part of trunk. Proboscis receptacle extends half to one third length of anterior trunk, never into posterior trunk. Lemnisci highly variable in shape and size, often coiled in anterior trunk or extending into posterior trunk.

Male (n = 11). Total length of everted cystacanth 3,496–4,305 (3,851). Proboscis 515–665 (581) long by 325–436 (388) wide. Proboscis hooks: I 72–104 (78), II 83–115 (98), III 84–106 (96), IV 83–112 (98), V 80–114 (99), VI 88–104 (91), VII 64–91(76). Proboscis receptacle 1,280–1,680 (1,533) long, 268–320 (291) wide. Neck 610–960 (839) long, 341–451 (396) wide. Anterior trunk 1,040–1,260 (1,135) long, 475–665 (553) wide; posterior trunk 1,160–1,460 (1,278) long, 594–820 (687). Somatic spines 12–14 (13) long, 6–8 (7) wide. Testes 2, ovoid, either both in anterior or posterior trunk or one in each part; anterior testis 164–248 (190) long, 94–128 (111) wide; posterior testis 154–240 (183) long, 98–138 (112) wide. Cement-glands not observed.

Female (n = 11). Total length everted cystacanth 3,718–4,254 (3,985). Proboscis 578–665 (611) long by 396–475 (440) wide. Proboscis hooks: I 83–106 (94), II 100–120 (110), III 80–118 (104), IV 86–114 (104), V 90–109 (102), VI 77–102 (91), VII 78–96 (87). Proboscis receptacle 1,440–1,780 (1,618) long, 268–364 (316) wide. Neck 660–860 (836) long, 396–515 (450) wide. Anterior trunk 1,100–1,240 (1,154) long, 540–689 (610) wide; posterior trunk 1,280–1,460 (1,384) long, 657–816 (754) wide. Somatic spines 12–14 (13) long, 6–8 (7) wide. Vagina with double sphincter.

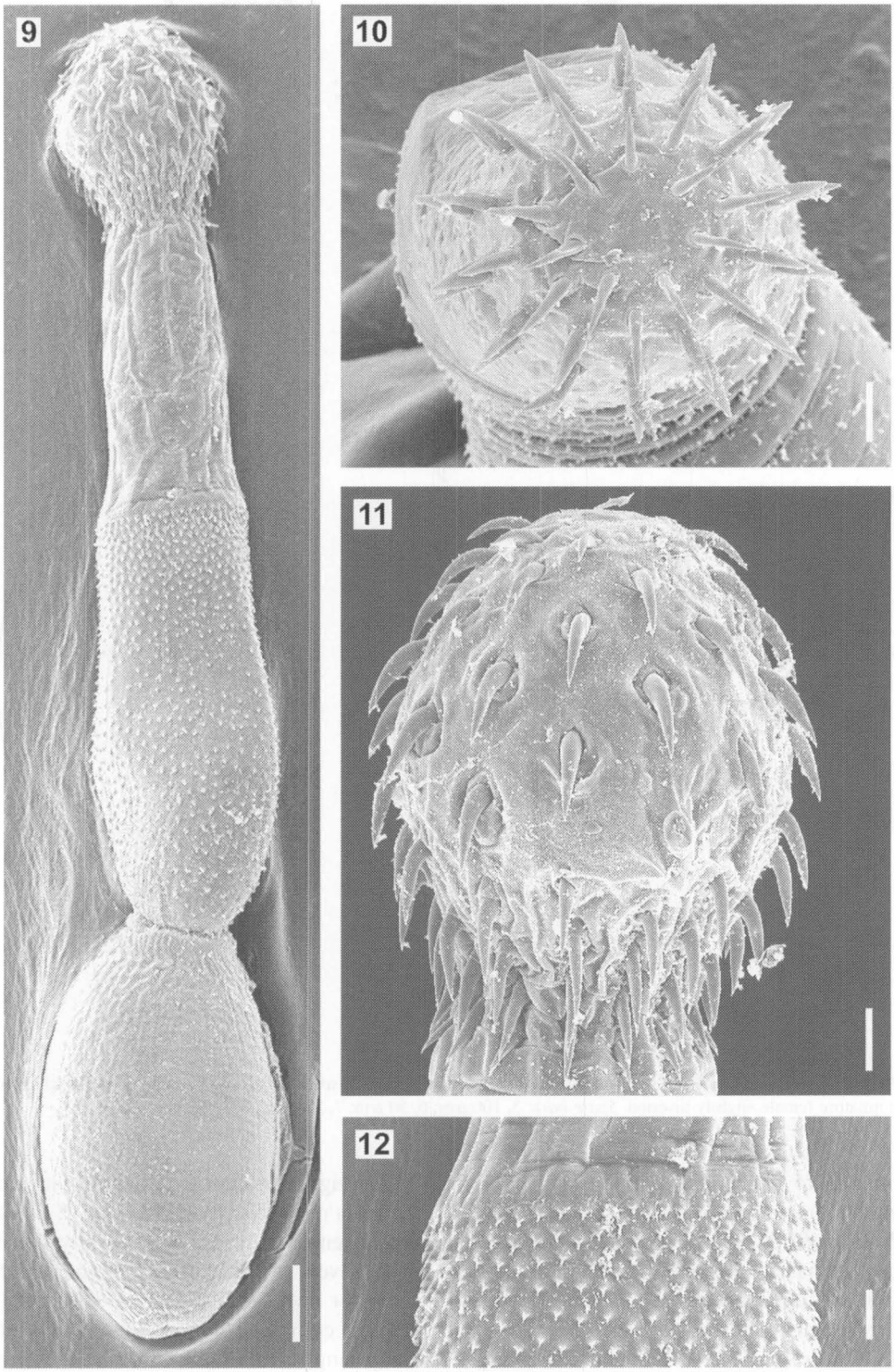
Remarks

The adult specimens had not been relaxed prior to fixation. Consequently probosces were not completely extended and posterior ends contracted and distorted. Nevertheless, sufficient morphological de-



Figures 5–8. Profilicollis novaezelandensis n. sp. from *Haematopus ostraglegus finschi*. 5. Proboscis, male; 6. Proboscis hooks; 7. Male; 8. Posterior end, immature female slightly inverted. *Scale-bars*: 5, 100 μm; 6, 50 μm; 7, 500 μm; 8, 200 μm.

tail was available to determine that the specimens here designated *P. novaezelandensis* n. sp. fall within the genus *Profilicollis* as resurrected by Nickol et al. (1999), because it has a subspherical proboscis, long neck, eggs without polar prolongations and decapod crustaceans as intermediate hosts. Using the key of Amin (1992), *P. novaezelandensis* falls into the group of species with an ovoid proboscis less than 1 mm in diameter. Of this group *P. novaezelandensis* differs from all, except *P. formosus* (Schmidt & Kuntz, 1967), in having: a proboscis armature with no more than 16 rows of hooks. It differs from *P. formosus* in having seven or eight per row, usually seven, compared with seven to nine, usually eight, for *P. formosus*; a longer neck, 952–1,190 (13% of total adult body length) compared with 773–900 (6% of total adult body length) for *P. formosus*; lemnisci in the males <3 mm long compared with >3 mm; and smaller eggs, 49–59 × 17–19 compared with 62–65 × 23. The tips of the thorns on the hooks of the proboscis of



Figures 9–12. Cystacanth of *Profilicollis novaezelandensis* n. sp. from *Hemigrapsus crenulatus*. 9. Cystacanth, everted; 10. Proboscis, *en face* view, note clear distinction of 14 longitudinal rows of hooks; 11. Proboscis, lateral view, note 7 hooks per row; 12. Small spines on anterior trunk. *Scale-bars*: 9, 200 μm; 10,11, 60 μm; 12, 40 μm.

P. novaezelandensis are recurved; those of *P. formosus* are not. *P. novaezelandensis* occurs in oystercatchers, godwits, and grapsid and ocypodid crabs in New Zealand, while *P. formosus* occurs in ducks and shrimps (*Macrobrachium* sp.) from Taiwan (Schmidt & Kuntz, 1967).

The three other species with at least16 rows of proboscis hooks all have more than 16 rows. *P. major* (Lundström, 1942) and *P. botulus* (Van Cleave, 1916) have 16–20 rows (Amin, 1992). *P. chasmagnathi* (Holcman-Spector, Mañe-Garzón, & Dei-Cas, 1977) has 16–20 rows (Holcman-Spector et al., 1977a,b), 16–18 rows (Vizcaino, 1989) or 18–20 rows (Martorelli, 1989), compared to 14–16 rows for *P. novaezelandensis*.

P. major and *P. botulus* both have a constriction of the trunk at the level of, or posterior to, the posterior end of the lemnisci, while *P. novaezelandensis* does not. Furthermore, *P. major* and *P. botulus* occur in a range of species of ducks from the Northern Hemisphere (Amin, 1992).

P. chasmagnathi has a wider proboscis, up to 2.11 mm, six to eight hooks per row, a subterminal genital pore and wider eggs (53 × 23 μm) in the female, and a posterior testis smaller than the anterior testis, compared with a proboscis of up to 0.65 mm, 7–8 hooks per row, a female with a terminal genital pore and narrower eggs (54 × 17 μm), and anterior and posterior testis of a similar size in *P. novaezelandensis* (see Vizcaíno, 1989).

The other *Profilicollis* species also found occurring in godwits and oystercatchers in New Zealand is *P. antarcticus*. *P. novaezelandensis* can be distinguished from *P. antarcticus* by having 14–16 longitudinal rows of hooks, compared to 18–22, and a smaller, subspherical proboscis of <0.7 mm rather than a larger, spherical proboscis of 1–2 mm in diameter (see Amin, 1992; Zdzitowiecki, 1985).

Although in this study the life-cycle has not been tested experimentally, it is believed that the cystacanths and adults described above belong to the same species, because they have the same number, size and arrangements of proboscis hooks.

Discussion

Polymorphus Lühe, 1911 has had a confusing taxonomic history (Amin, 1992) and the relationships between *Polymorphus* and the related genera *Arythmorhynchus* Lühe, 1911 (see Amin, 1992) and *Corynosoma* Lühe, 1904 (see Aznar et al., 1999) still require clarification. Using both morphological and life-history differences Nickol et al. (1999) reinstated *Profilicollis* Meyer, 1931 for polymorphids with long necks, fully ovoid probosces in both sexes, eggs with concentric membranes and decapods as intermediate hosts. Each of the presently known species has been previously included in this taxon either as a subgenus (Schmidt & Kuntz, 1967; Amin, 1985) or had been originally assigned to the genus *Profilicollis* or its synonym *Falsifilicollis* Webster, 1948 (see Nickol et al., 1999), resulting in ten, possibly nine (see possible synonym below), recognised species. These are: *P. altmani* (Perry, 1942), *P. antarcticus*, *P. arcticus* (Van Cleave, 1920), *P. botulus* (type-species), *P. chasmagnathi*, *P. formosus*, *P. kenti* (Van Cleave, 1947), *P. major*, *P. sphaerocephalus* and *P. texensis* (Webster, 1948) – possibly a junior synonym of *P. altmani* (see Karl, 1967, as cited in Nickol et al., 1999).

An additional species, *Polymorphus* (*Profilicollis*) *bullocki* Mateo, Córdova & Guzmán, 1982, which was previously overlooked in the literature, should also be included in *Profilicollis*, because it has the decapod *Emerita analoga* (Stimpson) as its intermediate host (Mateo et al., 1982, 1983) and the typical morphology of the genus. Together with *P. novaezelandensis* n. sp., this brings the number of species in the genus to 12 (possibly 11).

Although not confirmed by experimental infection, the link between the cystacanths found in the crabs and adults occurring in South Island pied oystercatchers and godwits is supported by both morphological and ecological evidence. South Island pied oystercatchers have been reported as feeding on crustaceans including the grapsid crab *Helice crassa* (see Baker, 1974). As the other two crabs, *Hemigrapsus crenulatus* and *Macrophthalmus hirtipes*, also occur in the mudflats together with *H. crassa* (A. Brockerhoff, pers. observation), it is not unlikely that the oystercatchers also occasionally eat them. Given that the marbled godwit has been reported feeding on *Hemigrapsus* spp. at a mudflat in California (Gratto-Trevor, 2000), it seems reasonable to suggest that the bar-tailed godwit may feed on the same genus of crabs in New Zealand. The mature and larval stages of *P. novaezelandensis* described in this study were collected from sites about 40 km apart. However, oystercatchers occur at the crab collection site and vice versa. Oystercatchers and crabs from both sites could therefore be the potential hosts for *P. novaezelandensis*. At the Avon-Heathcote Estuary, where *H. crassa* harbours *P. novaezelandensis*, none of the South Island

pied oystercatchers were found to be infested with *P. novaezelandensis*. This could be due to either the relatively low proportion of cystacanths of *P. novaezelandensis* in crabs from the Avon-Heathcote Estuary or the overall prevalence of Acanthocephala was very low in the oystercatchers from the Avon-Heathcote Estuary. A larger sample size may have included hosts infected with *P. novaezelandensis*.

Oystercatchers in this survey were found to harbour both *P. novaezelandensis* and *P. antarcticus*, as well as a *Plagiorhynchus* sp. *Profilicollis antarcticus*, first described from the South Shetlands, subsequently from Chile (Zdzitowiecki, 1985; Torres et al., 1991, 1992) and now from New Zealand, has not been reported from Australia. By contrast, *P. sphaerocephalus*, reported from Australia (Mawson et al., 1986) and Brazil (Yamaguti, 1963), was not found in this study. In each case this may reflect the lack of comprehensive surveys of possible host species. The scanty records of helminths and especially of acanthocephalans from New Zealand birds, summarised in the checklists of Weekes (1982) and more recently McKenna (1998), emphasise the lack of data. The New Zealand kingfisher *Halcyon sancta vagans* could, for example, be a host for *Profilicollis* sp., as it feeds almost exclusively on the mud crab *H. crassa* when it overwinters at coastal lagoons (Hayes, 1989).

Godwits, migratory visitors to New Zealand, were found in this survey to harbour both *P. novaezelandensis* and *P. antarcticus*, but not *P. sphaerocephalus*. *Profilicollis* spp. are not found in godwits frequenting Australian shores, which harbour *Arythmorhynchus limosae* Edmonds, 1971, a species not yet recorded from New Zealand hosts.

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Nectonema zealandica n. sp. (Nematomorpha: Nectonematoidea) parasitising the purple rock crab *Hemigrapsus edwardsi* (Brachyura: Decapoda) in New Zealand, with notes on the prevalence of infection and host defence reactions

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Abstract

A new species of marine hairworm, *Nectonema zealandica* (Nematomorpha: Nectonematoidea), is described from the purple rock crab *Hemigrapsus edwardsi* Hilgendorf from the South Island, New Zealand. This is the first record of *Nectonema* in the South Pacific Ocean and the southernmost locality for the genus. The description is based on juveniles and pre-adults taken from crabs. The new species is characterised by its stomal structure, presence of four cephalic papillae, mesenchyme arranged in eight lobes in pre-adults, insertion of muscle layer increasing body diameter at the septum and translucent anterior chamber. Data on the prevalence of infection over a three-year period, rates of parasitism in relation to host sex and size, and host defence reactions are presented. A list of all reported hosts of nectonematids is included.

Introduction

Marine hairworms of the genus *Nectonema* Verrrill, 1879 (Nectonematoidea) and phylum Nematomorpha are an enigmatic group of worldwide occurrence. They share the phylum with freshwater hairworms, both having similar morphological and biological features (obligate parasites of invertebrates). There are only five species of marine hairworms known (including the present one) and all develop in crabs and shrimps (Table 1). The present paper describes a new species of marine hairworm parasitising the brachyuran crab *Hemigrapsus edwardsi* Hilgendorf (Decapoda: Grapsidae) from the South Island, New Zealand and discusses the prevalence of infection over a three year period. This is the first definite record of *Nectonema* in the South Pacific Ocean, some 12,000 km from the nearest recorded site (Indonesia) and the southernmost locality for the genus. An early possible record of this occurrence may have been made by von Linstow (1888) when he mentioned 'a *Gordius* found in a crab dredged near New Zealand'. However no description accompanied this note and there is no way to say whether this report refers to *Nectonema* or a member of the phylum Nematoda, several genera of which occur in crabs worldwide.

Materials and methods

Specimens of *Hemigrapsus edwardsi* were collected from a rocky intertidal beach in Canterbury, South Island, New Zealand (172°53′E., 43°06′S.) over a three year period. During the course of this study, a total of 636 crabs (447 females and 189 males) were dissected (Table 2). Females were selectively chosen since the study was an investigation on the effects of parasitism on crab reproduction. Most samples were collected during the first five months of the years 1998, 1999 and 2000 (Table 2). The crabs were maintained in the laboratory for one to several weeks (some up to 12 weeks) in tanks of running seawater at 12-15° C and fed blue mussels (*Mytilus edulis*) two or three times a week, eventually undergoing dissection after being killed by freezing. The number, position and condition of the parasites together with the size (carapace width,

Table 1. Hosts (crabs and shrimps) of *Nectonema* species (only the earlist reference to each specific host-parasite association is listed) (modified and updated from Nielsen, 1969).

Host	*Nectonema* species	Reference
DECAPODA		
Dendrobranchiata		
Amalopenaeus elegans (Smith)	*N. agile*	Bate, 1888
Sergestes arcticus Krøyer	*N. munidae*	Greve, 1972
Pleocyemata		
Caridea		
Lebbeus polaris (Sabine)	*N. agile*	Nouvel & Nouvel, 1938
Palaemon serratus (Pennant)	*N. agile*	Nouvel & Nouvel, 1934
P. elegans Rathke (= *Leander squilla* (L.))	*N. agile*	Nouvel & Nouvel, 1938
Palaemonetes sp.	*N. agile*	Ward, 1892
P. vulgaris Say	*N. agile*	Born, 1967
Pandalus borealis Krøyer	*N. agile*	Leslie et al., 1981
Pandalus montagui Leach	*N. agile*	Skaling & MacKinnon, 1988
Pontophilus norvegicus (M. Sars)	*N. munidae*	Brinkmann, 1930
Anomura		
Anapagurus hyndmanni (Thompson)	*N. agile*	Perez, 1927
A. laevis (Bell)	*N. agile*	Perez, 1934
Diogenes pugilator (Roux)	*N. agile*	Pérez, 1934
Munida sarsi (Brinkmann)	*N. munidae*	Nielsen, 1969
Munida tenuimana G. Sars.	*N. munidae*	Brinkmann, 1930
Pagurus acadianus Benedict	*N. agile*	Leslie et al., 1981
P. bernhardus (L.)	*N. agile*	Mouchet, 1931
P. cuanensis Thompson	*N. munidae*	Nielsen 1969
P. pubescens Krøyer	*N. munidae*	Brinkmann, 1930
Brachyura		
Cancer borealis Stimpson	*N. agile*	Leslie et al., 1981
C. irroratus (Say)	*N. agile*	Leslie et al., 1981
Erimacrus isenbeckii	?	Oku et al., 1983
Hemigrapsus edwardsi Hilgendorf	*N. zealandica* n. sp.	Present paper
Macropipus arcuatus (Leach)	*N. agile*	Gallien, 1949
M. pusillus (Leach)	*N. agile*	Pérez, 1927
Macropodia rostrata (L.)	*N. agile*	Pérez, 1927

CW) and gender of the hosts, were recorded. Most crabs had a CW greater than 24 mm, indicating that there were mature at the time of collection. The parasites used for the description were preserved in alcohol and processed to glycerine for microscopical observations. Cross-sections of the parasites and capsules surrounding the parasites were made by hand with a razor blade. Measurements are given in micrometres except where indicated.

Results

The nectonemids were usually curled up beside or under the midgut and posterior midgut caecum; however, some occurred under the carapace adjacent to the membrane lining the branchial chamber (Figure 1). From a total of 636 crabs dissected, 81 (12.7%) were infected with *Nectonema*. The monthly parasitism rates for male and female crabs over the three year period varied between 0 and 31.6% with females having generally a higher rate of parasitism than males (Figure 2). Infection rates were 0–27% in different crab

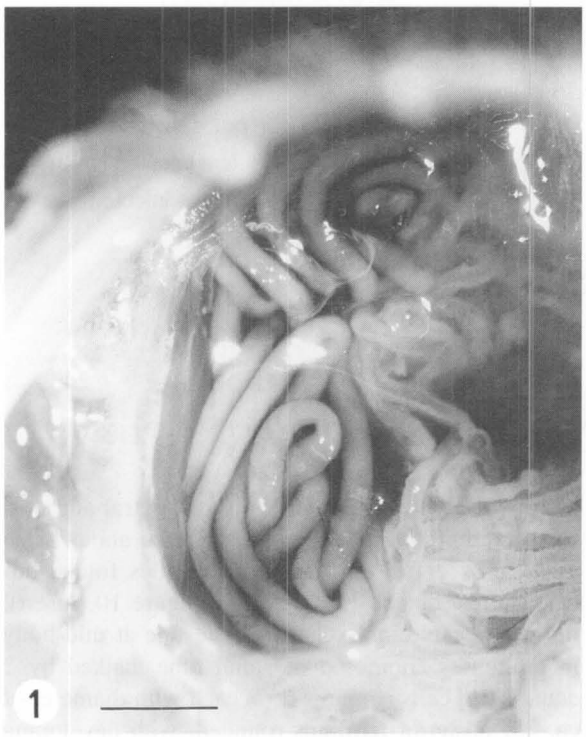


Figure 1. Nectonema zealandica n. sp. in the body-cavity (hepatopancreas) of *Hemigrapsus edwardsi*. *Scale-bar*: 3 mm.

Table 2. Numbers of male and female *H. edwardsi* examined (males/females) over the three-year study period.

Month	1998	1999	2000
January	44/100	–	–
February	50/57	0/5	–
March	9/72	0/18	32/0
April	10/68	–	44/19
May	–	–	0/93
December	0/15	–	–
Total	425 (113/312)	23 (0/23)	188 (76/112)

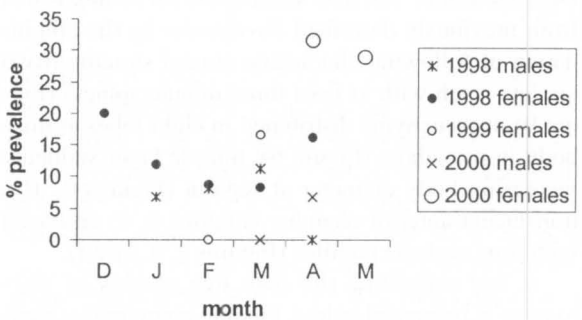


Figure 2. Monthly prevalence of *Nectonema zealandica* n. sp. in males and females of *Hemigrapsus edwardsi* from 1998 to 2000.

sizes, being higher in females (Figure 3). Female parasitism rates increased with carapace width until they reached a maximum in the 40–44 mm group (27%), after which the rate decreased for the largest females (Figure 3). Male parasitism rates never exceeded 10% and varied little relative to size (Figure 3). Normally, one nectonematid was found in each host (91.4%); however, in seven cases, multiple infections occurred (two parasites four times, three parasites twice and four parasites once). No pathological effects of the parasites on their hosts were noted.

The parasites were in various developmental stages within their hosts (from parasitic juvenile [stage undergoing development] to mature juvenile [development essentially completed] or pre-adult [juvenile ready to or in the process of moulting to the adult stage]). Mortality rates, based on the external appearence of the parasites in crab hosts, was high with dead or dying parasites in various stages of encapsulation. Only 4.3% of the infected crabs contained unencapsulated developing parasites. Specimens used in the present description (which ranged in length from 13 to 300 mm) were all females and included two pre-adults (corresponding to the LA stage of Schmidt-Rhaesa, 1997) and four parasitic juveniles (corresponding to the L stage of Schmidt-Rhaesa, 1996). Morphological characters in the anterior end of pre-adults and adults are essentially similar (Schmidt-Rhaesa, 1997). Since the pre-adults still possessed their juvenile cuticles, details of the stoma could be observed. However the natatory bristles on the adult cuticle were folded under the juvenile cuticle and could not be characterised. A comparison with previously described species of *Nectonema* indicated that the present specimens were new and a description follows.

Nematomorpha Vejdovsky, 1886
Nectonematoidea Rauther, 1930
Nectonema Verrill, 1879

Nectonema zealandica n. sp.

Description (Figures 4–12)

Living worms removed from hosts were white to cream colour and soft in texture; 13 (parasitic juvenile) to 300 mm (pre-adult) in length and 472 (parasitic juvenile) to 737 (pre-adult) in greatest width; length from head to septum, 252 (parasitic juvenile) to 575

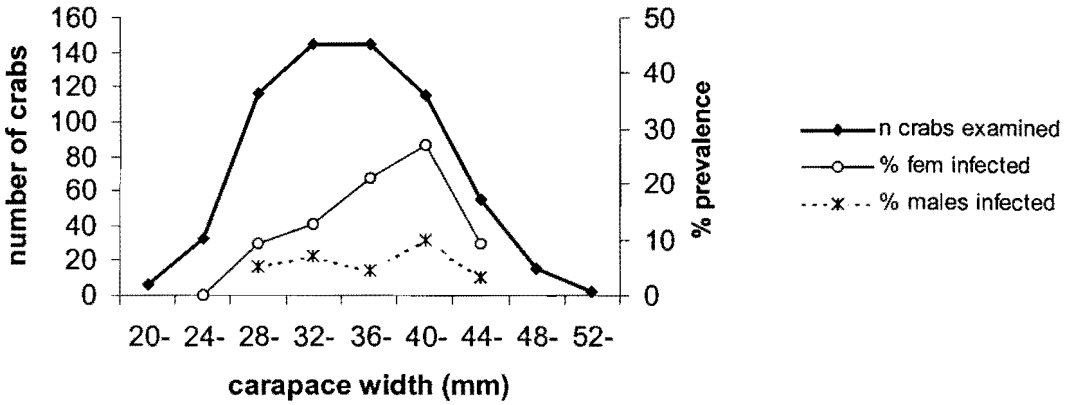


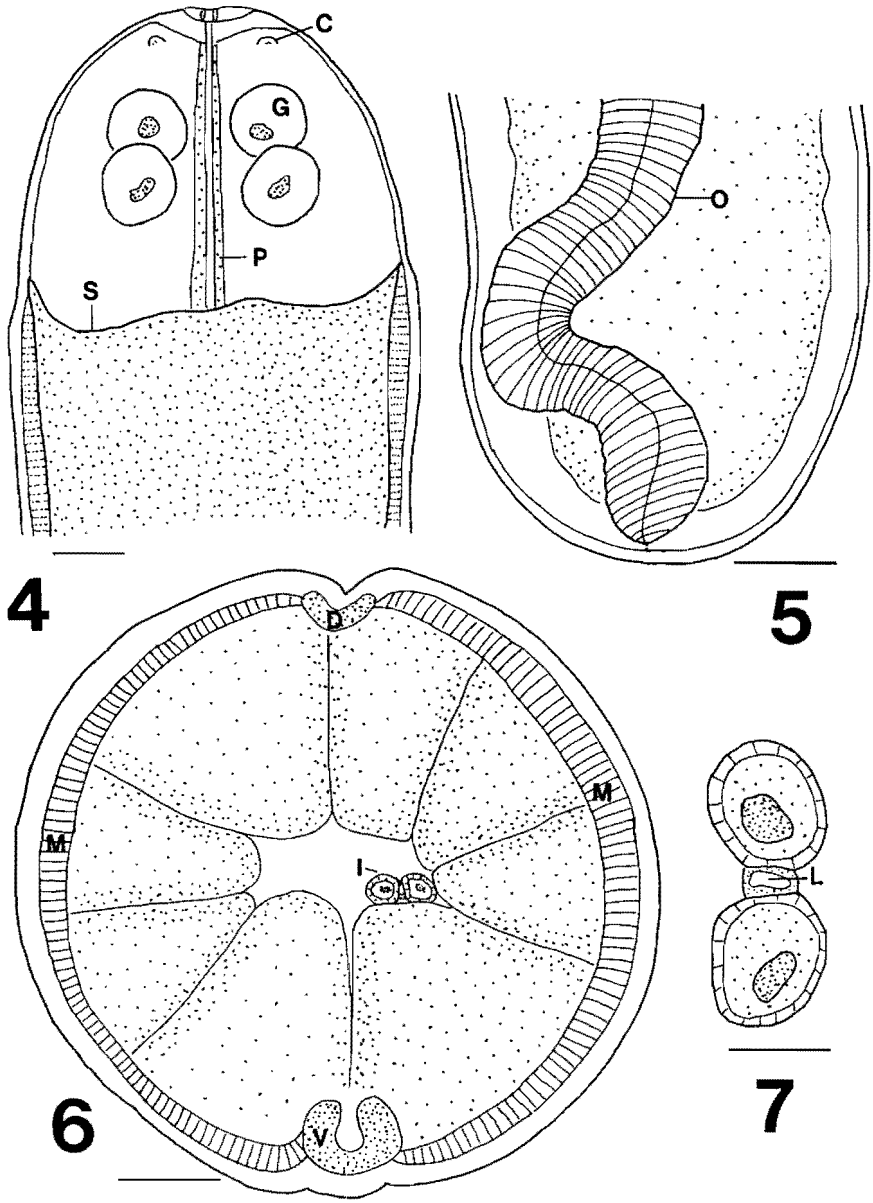
Figure 3. Size distribution of *Hemigrapsus edwardsi* related to the prevalence of *Nectonema zealandica* n. sp.

(pre-adult); septum not always distinct in parasitic juveniles; diameter of head at septum, 296 (parasitic juvenile) to 554 (pre-adult), diameter at posterior end, 164 (parasitic juvenile) to 460 (pre-adult); anterior chamber in all stages with 4 giant cells (Figures 4, 8) from 88 to 126 in diameter; mouth opening in slight depression in centre of head (Figure 4); stoma (portion of alimentary tract between mouth opening and pharynx) (Figure 9) composed of sclerotised irregular tube 38 to 41 long and 26 to 28 at widest point; containing 2 circlets of at least 3 spines protruding from inner walls; anterior set located just posterior to mouth opening with spines ca. 3.5 in length; second set located slightly posterior to first with spines ca. 3 in length; head with 4 faint papillae spaced almost equidistant around mouth opening (Figure 9, insert); each papilla ca. 28 in diameter at base and ca. 7.5 in height; pharynx a straight, narrow tube surrounded by thin tissue layer, extending from base of stoma (Figure 4) through centre of septum; cuticle thin in parasitic juveniles, 1.5 to 3.0 at mid-body; sensory bristles scattered sparsely over juvenile cuticle (Figure 12); adult cuticle (under juvenile cuticle in pre-adults), 3.5 to 9.5 thick at mid-body; hypodermal cell layer thickness at mid-body 6 (parasitic juveniles) to 19 (pre-adults); longitudinal muscle layer begins at septum in older parasitic juveniles and pre-adults (Figures 4, 10), causing body width to suddenly increase at that point; muscle layer at mid-body, 29 (parasitic juveniles) to 34 (pre-adults) thick; cross-section at mid-body (Figure 6) in pre-adults shows mesenchyme (also called gono-parenchyma) almost filling body-cavity and separated into 8 lobes; mesenchyme composed of cells ranging of 12 to 18 in diameter; cells contain numerous spherical granules; body with depressions at regions of ventral and dorsal nerve cords (Figure 6); ventral cord about twice as large as dorsal cord; natatory bristles folded under juvenile cuticle in pre-adults (Figure 10, insert), most noticeable mid-ventrally; intestine at mid-body in pre-adults composed of small tube flanked by 2 equal-sized cells (Figures 6, 7) each with diameter of ca. 35; posterior terminus rounded, with developing gonopore located in centre of tail tip; oviduct muscular (Figure 5).

Many developing parasites were surrounded by capsules formed by host blood-cells (Figure 13). These capsules varied in length from 8 to 15 mm and in width from 1 to 6 mm. They ranged in colour from clear to various shades of yellow and brown, lighter coloured ones being translucent while darker ones were opaque.

Holotype: Pre-adult female deposited in the National Museum, Wellington, New Zealand.
Type-host: *Hemigrapsus edwardsi* Hilgendorf (Grapsidae: Brachyura: Decapoda: Crustacea).
Type-locality: Canterbury, South Island, New Zealand.
Differentiation. The new species can be distinguished from previously described *Nectonema* by the combination of following characters; stomal structure (two circlets, each with at least three minute spines) (Figure 9), mesenchyme distributed in eight lobes at mid-body in pre-adults (Figure 6), muscle layer suddenly increasing body diameter at septum (Figures 4, 10), translucent anterior chamber (Figures 4, 8) and head with four cephalic papillae (Figures 4, 9, insert).

A key separating the now five species of *Nectonema* is presented below. Finding comparative characters to separate species is difficult, since not all



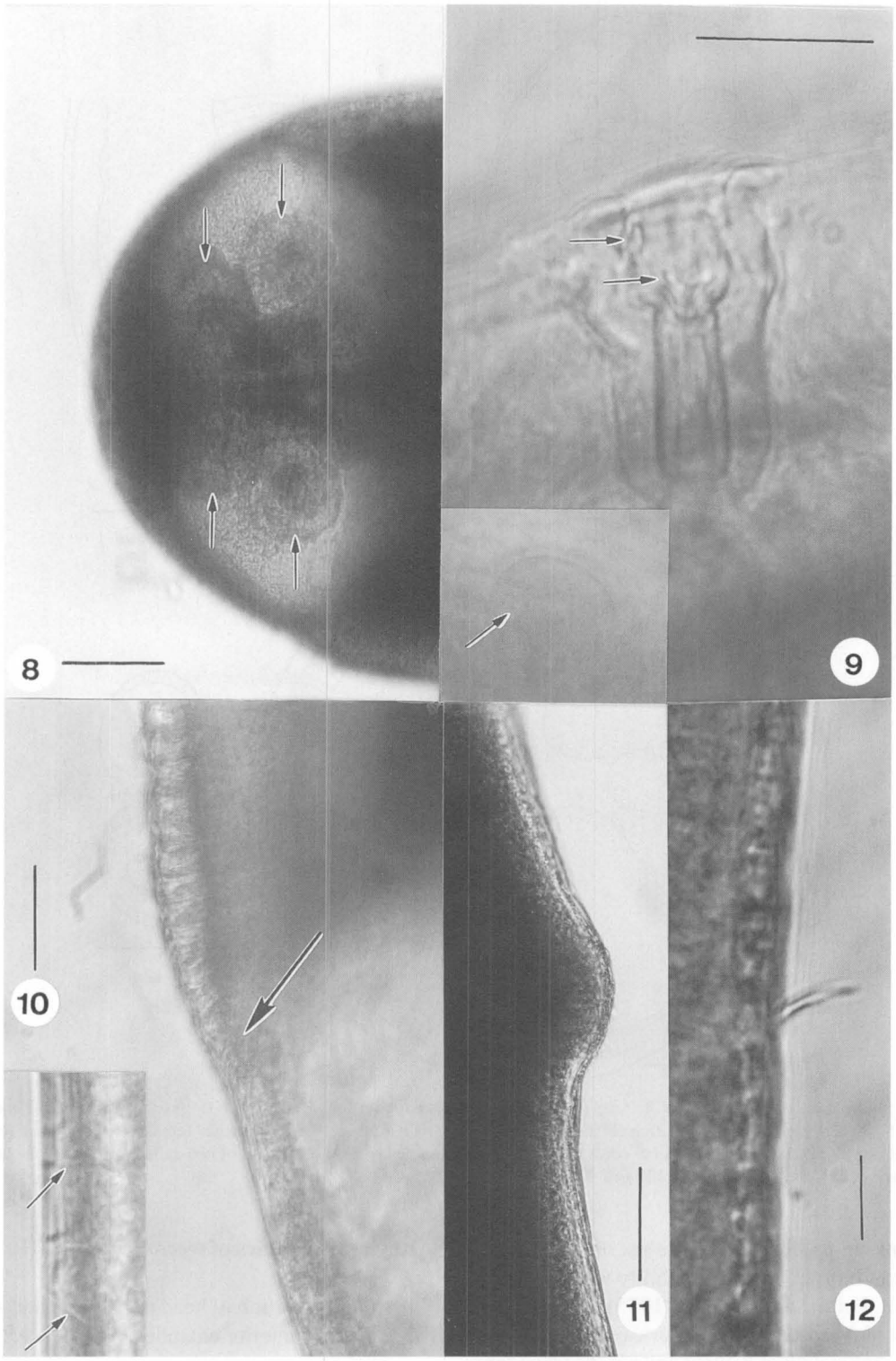
Figures 4–7. Nectonema zealandica n. sp. 4. Anterior chamber of pre-adult with four giant cells (G), pharynx (P), cephalic papilla (C) and septum (S). 5. Posterior end of pre-adult female showing oviduct (O). 6. Cross-section of pre-adult female showing eight sections to the mesenchyme, dorsal (D) and ventral (V) nerve cords, intestine (I) and muscle layer (M). 7. Detail of two-celled intestine with enclosed lumen (L). *Scale-bars*: 4, 197 μm; 5, 121 μm; 6, 121 μm; 7, 31 μm.

descriptions are based on the same sex or stage. Also, the degree of morphological variability within species is unknown. For instance, Bakke (1975) characterised specimens of *Nectonema* from Norway as having the anterior chamber with only three and not the usual four giant cells. Whether this represents a significant diagnostic character or variation within a population of *N. munidae* is unknown.

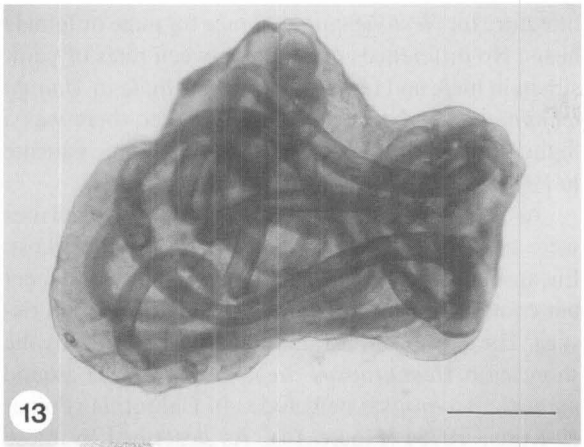
Key to the species of *Nectonema*

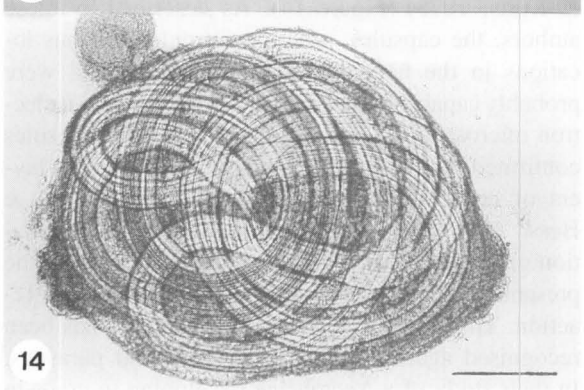
1 Anterior portion of head translucent and set off into a distinct anterior chamber 3

– Anterior portion of head dark, without distinct anterior chamber 2



Figures 8–12. Nectonema zealandica n. sp. 8. Head of parasitic juvenile showing four giant cells (arrows), two of which are partly obscured by other nerve-cells. 9. Stoma of pre-adult. Arrows show two rows of spines. (Insert shows cephalic papilla (arrow shows fine lines radiating from base). 10. Body-wall of pre-adult in region of septum showing sudden increase in body width as a result of insertion of the muscle band (arrow). Insert shows natatory bristles (arrows) folded under the juvenile cuticle. 11. Cyst on body wall of juvenile. 12. Body wall of parasitic juvenile with sensory bristle. *Scale-bars*: 8, 100 μm; 9, 20 μm; 10, 50 μm; 11, 95 μm; 12, 12 μm.





Figures 13–14. Host reactions against crab parasites. 13. Dead parasitic juvenile of *Nectonema zealandica* n. sp. surrounded by host capsule in *Hemigrapsus edwardsi*. (New Zealand). 14. Parasitic juvenile of *Ascarophis* sp. (Nematoda: Spirurida) surrounded by host capsule in *Hemigrapsus oregonensis*. (California, USA). *Scale-bars*: 13, 1.5 mm; 14, 440 μm.

2 Cuticle at mid-body 4 μm or greater in thickness; sensory bristles present on body surface; cuticular tubercles absent; rows of natatory bristles arranged more or less in single rank .... *N. svensksundi* Bock, 1913 (Norway)

– Cuticle at mid-body less than 3 μm in thickness; sensory bristles absent but minute, oblong; cuticular tubercles present on body surface; rows of natatory bristles arranged irregularly in several rank ... *N. melanocephalum* Nierstrasz, 1907 (Indonesia)

3 Body diameter anterior to septum less than posterior; stoma of pre-adult with two circlets of three spines each .... *N. zealandica* n. sp. (South Island, New Zealand)

– Body diameter anterior and posterior to septum approximately equal; stoma of pre-adult variable or with a single circlet of two spines 4

4 Male with scales on posterior portion of body; stoma of pre-adult variable; gradual torsion of anterior third of body results in natatory bristles on posterior two-thirds of body in a lateral position ..... *N. agile* Verrill, 1879 (Eastern North America, Western Europe, Mediterranean, Black Sea)

– Male without scales; stoma of pre-adult with a single circlet of two spines; natatory bristles located on dorsal and ventral sides of body throughout ..... *N. munidae* Brinkmann, 1930 (Norway)

Discussion

The shape, size, location and number of cephalic papillae can be important diagnostic characters (Figures 4, 9, insert), although they are very difficult to see, especially in the developing juveniles. The fine lines radiating from the base of each papilla are probably nerves and suggest a sensory nature for these structures (Figure 9, insert). Aside from the present study, head papillae have been observed only by Ward (1892) in *N. agile*. He stated, 'the head is marked anteriorly by the presence of a shallow median dorsiventral furrow, on each lateral edge of which are one, sometimes two low rounded papillae.' The papillae were on the underlying adult cuticle and most pronounced in the pre-adult forms.

The increase in body diameter at the septum as a result of the insertion of the longitudinal muscle band at that point is a curious feature of *N. zealandica*. This condition does not appear to exist in the previously described species.

The small intestinal lumen flanked by a pair of cells has been noted in other nectonematids. Ward (1892) commented that the intestine of *N. agile* was composed of four, three or two cells depending on the location in the body cavity. The same condition was noted by Skaling & MacKinnon (1988) in a species of *Nectonema* (possibly *N. agile*) collected off New Brunswick. In the present study, the cell walls were surprisingly thick and distinct, as was noted by Ward (1892) in *N. agile*, who even considered that the cells might be cuticularised. A similar two-walled intestine was also described by Nierstrasz (1907) for *N. melanocephalum*. Both a digestive and secretory function for the intestine have been suggested (Skaling & MacKinnon, 1988). Brescani (1991) compared the intestine of *Nectonema* with that of mermithid nematodes regarding its purpose as an uptake and storage organ. Actually, the intestine of nectonemids more

closely resembles the stichosome (modified pharynx) of mermithids in consisting of a narrow lumen surrounded by tissue (and containing a series of glandular stichocytes in mermithids) (Poinar, 1983). In *Nectonema*, the pharynx connects with the intestine, which is the case only with pre-parasitic stages of mermithids. As development proceeds in the latter, the intestine separates from the pharynx and develops independently into a large food storage organ, the trophosome, which corresponds functionally with the mesenchyme in nectonemids.

Another important diagnostic character that could be used to distinguish *Nectonema* spp. is the structure of the stoma (also called pre-pharyngeal or buccal region) in parasitic and pre-adult juveniles. The two circlets of spines on the stomal walls (Figure 9) may represent the spines on the proboscis of the infective juvenile and the shape, size and placement of these could vary for different species. In the only description of a *Nectonema* pre-infective juvenile, Huus (1932) described two rows of spines on the eversible proboscis of *N. munidae* G. Sars. If the spines on the cuticle of the pre-adult are carried over from the infective stage, it would suggest that no moult occurred during parasitic development since these spines would presumably have been shed. This would indicate that only a single pre-emergence moult occurs in the life-cycle of these forms, as was suggested by Schmidt-Rhaesa (1996).

Two swellings near the posterior end occurred on the body-wall of a developing juvenile in the present study (Figure 11). These enlargements contained dense bodies resembling cysts. In one were spherical bodies ca. 10 μm in diameter and adjacent to the cyst on the surface of the cuticle was an elliptical spore-like body ca. 11 μm in length. Ward (1892) identified sporozoites of a gregarine protozoan in the reproductive system and body wall of male *N. agile*. The cysts observed here could represent gregarine gametocysts with developing sporozoites. If these are parasitic gregarines, the *Nectonema* could have acquired the infection from parasitised crabs, since the latter are susceptible to gregarine infections which they in turn acquire from feeding on infected molluscs (Sparks, 1985).

In the present study, female crabs usually had higher monthly parasitism rates than males. As males and females of *H. edwardsi* inhabit the same area and have similar feeding habits, one would expect that they would be exposed to the same number of infective stages. There seems to be no pattern in the literature for *Nectonema* preference for male or female hosts. No differences occurred between rates of parasitism in male and female hosts for *N. agile* in *Munida tenuimana* (see Nielsen, 1969); however, there was a higher infection rate in male hosts of the same parasite in *Pagurus bernhardus* (see Mouchet, 1931).

As noted in the present study, many of the parasites were encapsulated in the body-cavity of their host. Encapsulated worms appeared somewhat translucent but contained the normal arrangement of internal tissues. The capsules were similar to those formed by the shore crab *Hemigrapsus oregonensis* (Dana) around parasitic *Ascarophis* nematodes in California (Poinar & Kuris, 1975) (Figure 14). As described by these authors, the capsules, which occurred in various locations in the host, could become thick and were probably capable of destroying the nematode. An electron microscopical study of these nematode capsules confirmed that they were formed by concentric layers of crab haemocytes or muscle cells (Poinar & Hess, 1977). Capsules composed of an accumulation of host cells surrounding dead *Nectonema* in the present study represent a very successful defence reaction. This is the first time a host reaction has been recognised against developing *Nectonema* parasites. In their study of a *Nectonema* developing in crabs in the Bay of Fundy, Leslie et al. (1981) stated, 'Brittle, transparent brown tubes, which were tentatively identified as shed cuticles, were present in some crabs.' These brown tubes were probably host melanisation reactions against the developing parasites as was noted against some nematode parasites in crabs (Poinar & Kuris, 1975) and not evidence of moults, as suggested by Leslie et al. (1981) and Bresciani (1991).

The high rate of encapsulation suggests that *H. edwardsi* is not the preferred host of *N. zealandica* since, in long-term parasitic associations, lethal host reactions are rare. A survey of other infected marine crustaceans in the area has not yet been undertaken, however the possibility of their existence is good, since *N. agile* and *N. munidae* are not host specific and multiple host genera are known for these species (Table 1). Most recorded hosts of nectonematids are crab-like (Anomura and Brachyura) with the remainder shrimps (Dendrobranchiata and Caridea) (Table 1).

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